Harvard Medical School

Comparative Pathology

and

Tropical Medicine

PLATE I

	I. **PALE YELLOW.**
	II. **LIGHT YELLOW.**
	III. **YELLOW.**
	IV. **REDDISH YELLOW.**
	V. **YELLOWISH RED.**
	VI. **RED.**
	VII. **BROWNISH RED.**
	VIII. **REDDISH BROWN.**
	IX. **BROWNISH BLACK.**
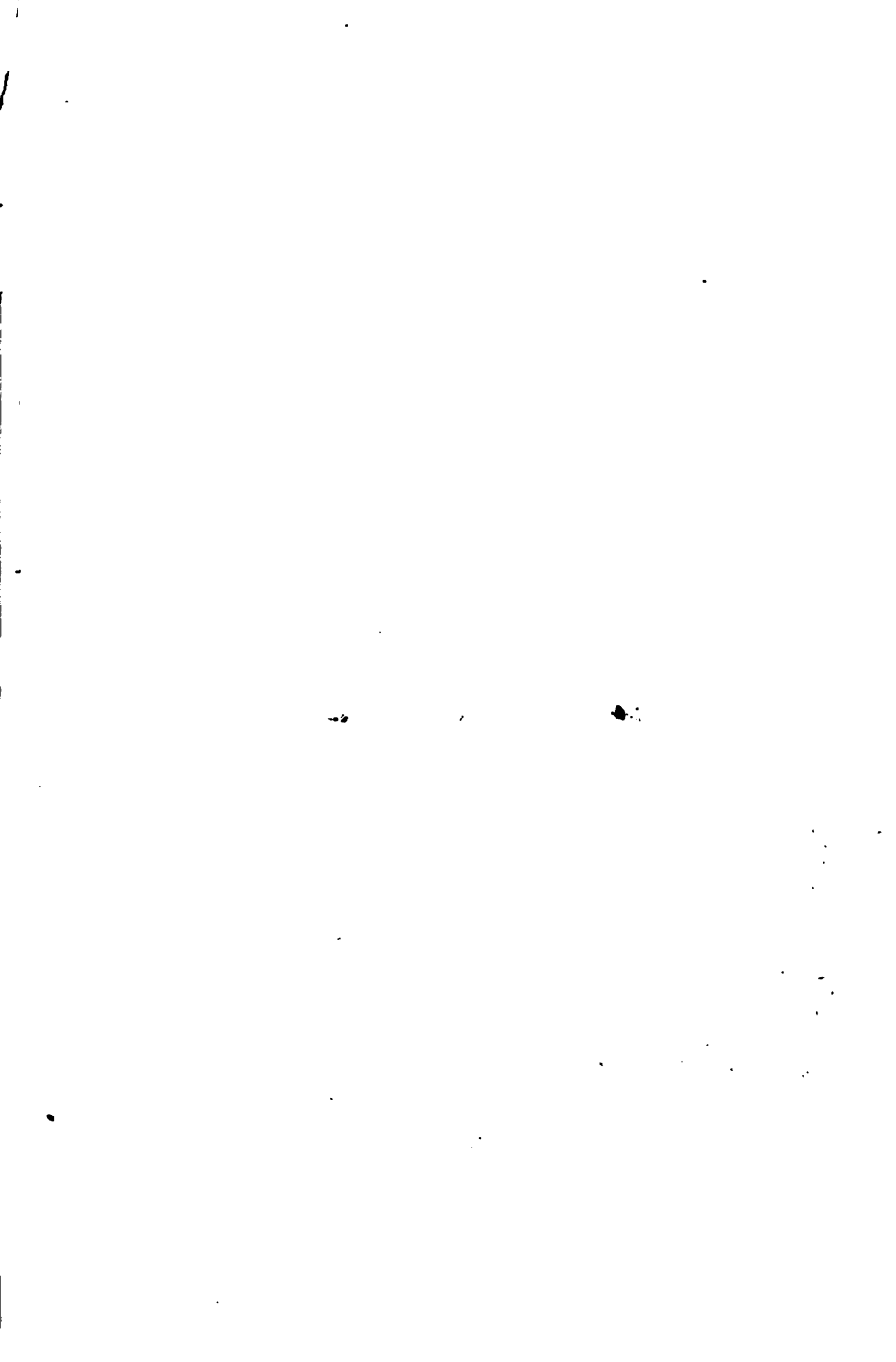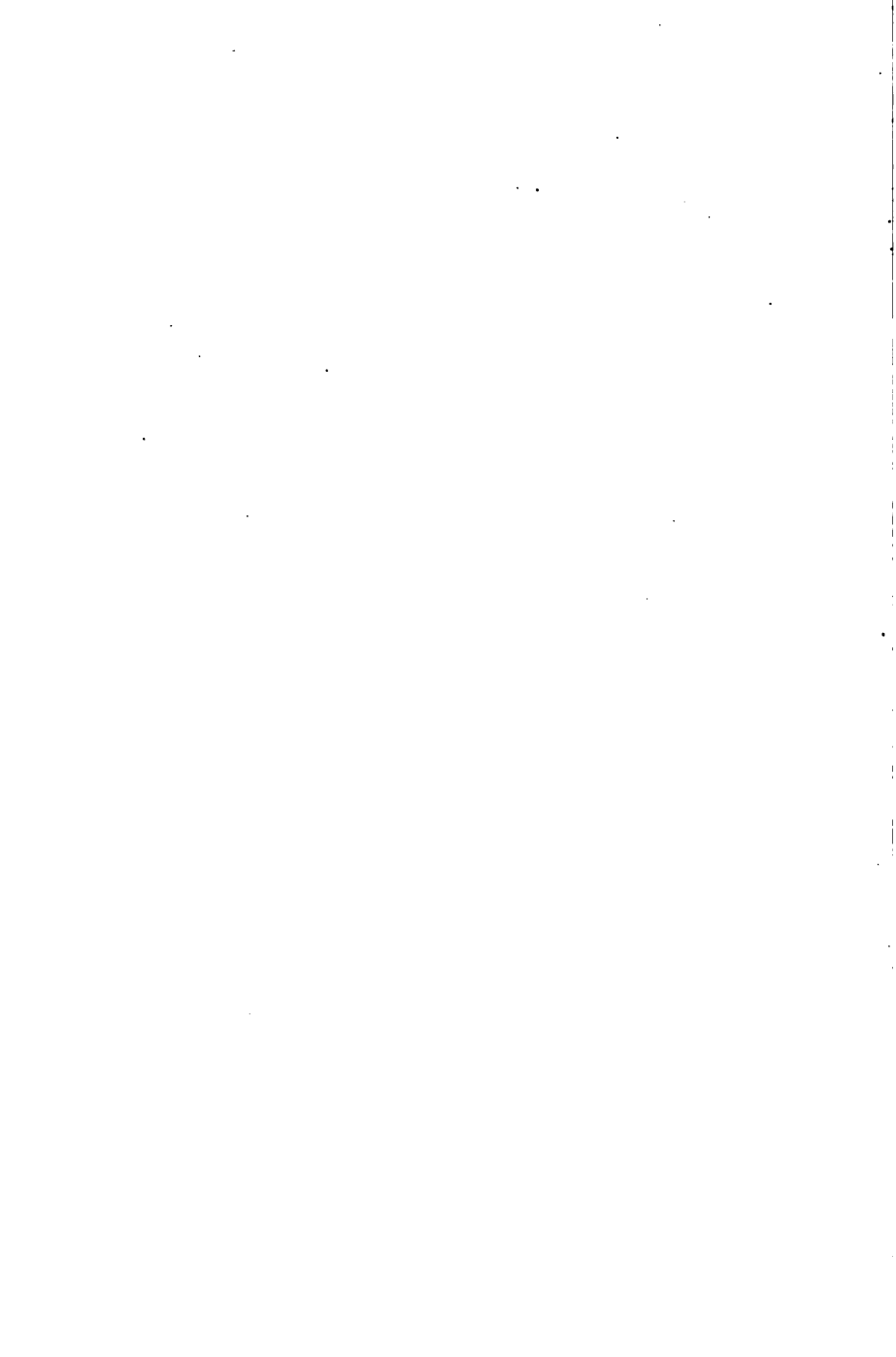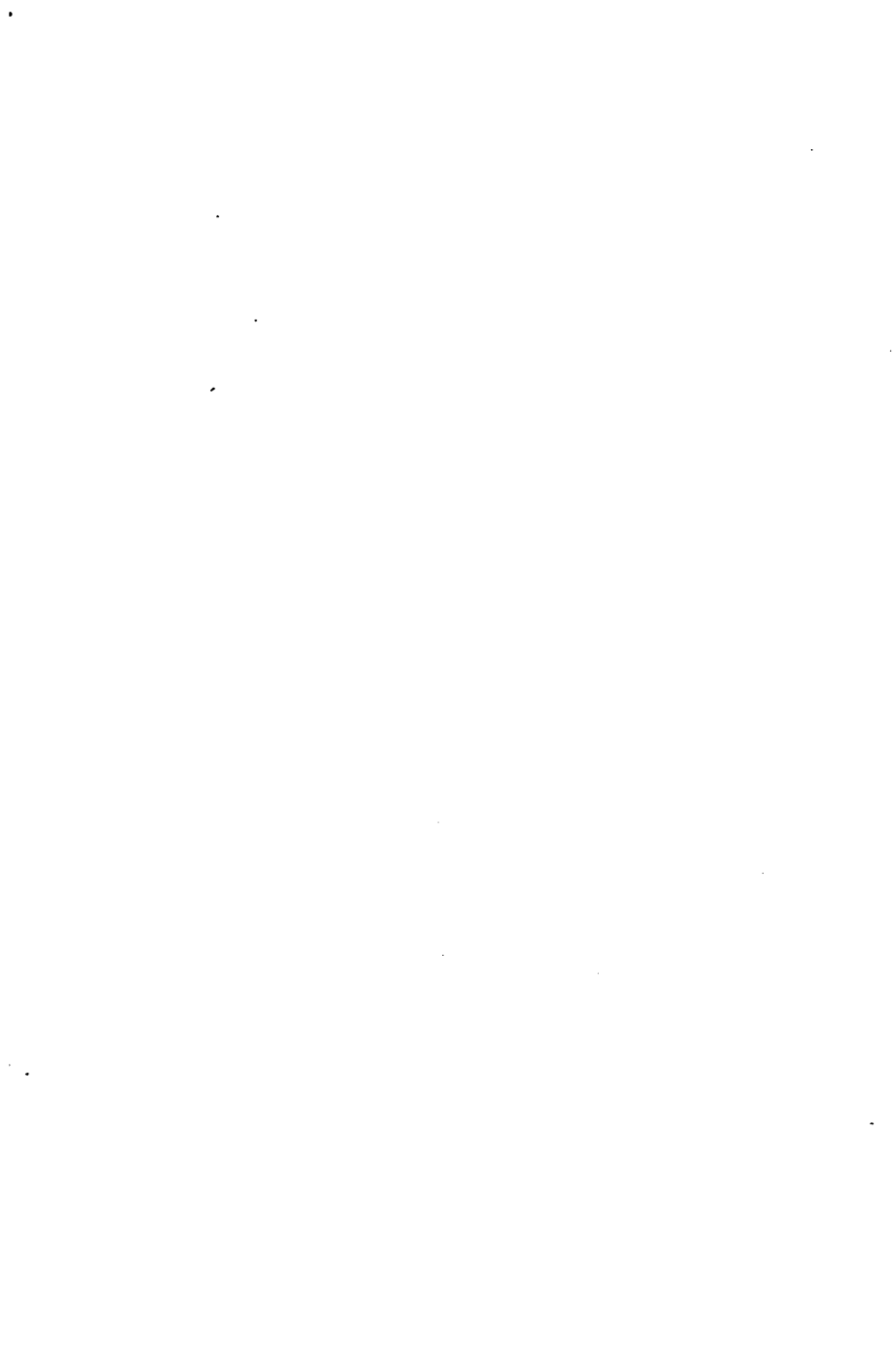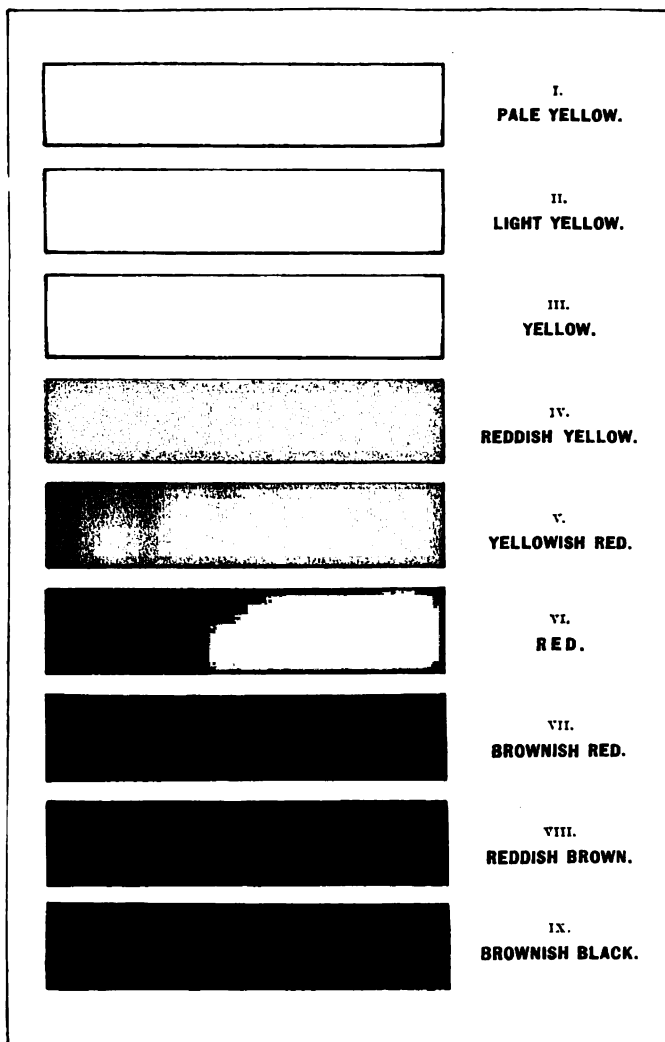
Scale of urinary colors, according to Vogel.

Clinical Diagnosis

A MANUAL OF LABORATORY METHODS

BY

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Illustrated

Second Edition, Revised and Enlarged

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TO

MY FATHER

Joe H. Todd, M. D.

THESE PAGES ARE

AFFECTIONATELY DEDICATED

PREFACE TO THE SECOND EDITION

WHILE the original purpose of this book—to present clearly and concisely the various laboratory methods which are of use in clinical medicine—has not been lost sight of, its scope has been somewhat enlarged in the present edition.

Each section has been carefully revised and much new material has been added to every chapter. Among the many additions may be mentioned: the use of artificial light and the importance of numerical aperture in microscopic work; photomicrography with simple apparatus; the antiformin method for tubercle bacilli; detection and significance of albumin in the sputum; Tsuchiya's modification of Esbach's test; the formalin test for ammonia and Benedict's methods for sugar in urine; volume index of red blood-corpuscles; Wright and Kinnicutt's method of counting blood-platelets; Harlow's blood-stain; a simple technic for the diagnosis of typhoid fever by blood-cultures; the Wassermann reaction, and Frothingham's impression method in the diagnosis of rabies.

Because of the growing importance of animal parasites, this chapter has been entirely rewritten and more than doubled in extent. Two new chapters have been added: one upon Bacteriologic Methods, which supplements the methods given in other portions of the book, and one upon Preparation and Use of Vaccines, including therapeutic and diagnostic use of tuberculin.

Some of the illustrations have been replaced with better ones and many new pictures have been added, including eight photomicrographs by Dr. W. P. Harlow, and a considerable number by the author. Through courtesy of Dr. Langdon Frothingham, of Harvard University, a colored plate showing Negri bodies as seen in his impression method has been included.

The author wishes to express his indebtedness to Francis Ramaley, Ph.D., Professor of Biology in the University of Colorado, and T. D. A. Cockerell, Professor of Systematic Zoology, for suggestions as to the nomenclature of animal parasites; and to Dr. A. R. Peebles, Professor of Medicine, for suggestions and aid throughout the revision.

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PREFACE

THIS book aims to present a clear and concise statement of the more important laboratory methods which have clinical value, and a brief guide to interpretation of results. It is designed for the student and practitioner, not for the trained laboratory worker. It had its origin some years ago in a short set of notes which the author dictated to his classes, and has gradually grown by the addition each year of such matter as the year's teaching suggested. The eagerness and care with which the students and some practitioners took these notes and used them convinced the writer of the need of a volume of this scope.

The methods offered are practical; and as far as possible are those which require the least complicated apparatus and the least expenditure of time. Simplicity has been considered to be more essential than absolute accuracy. Although in many places the reader is given the choice of several methods to the same end, the author believes it better to learn one method well than to learn several only partially.

More can be learned from a good picture than from any description, hence especial attention has been given to the illustrations, and it is hoped that they will serve truly to *illustrate*. Practically all the microscopic struc-

tures mentioned, all apparatus not in general use, and many of the color reactions are shown in the pictures.

Although no credit is given in the text, the recent medical periodicals and the various standard works have been freely consulted. Among authors whose writings have been especially helpful may be mentioned v. Jaksch, Boston, Simon, Wood, Emerson, Purdy, Ogden, Ewald, Ehrlich and Lazarus, Da Costa, Cabot, Osler, Stengel, and McFarland.

The author wishes hereby to express his indebtedness to Dr. J. A. Wilder, Professor of Pathology in the Denver and Gross College of Medicine, for aid in the final revision of the manuscript; and to W. D. Engel, Ph.D., Professor of Chemistry, for suggestions in regard to detection of drugs in the urine. He desires to acknowledge the care with which Mr. Ira D. Cassidy has made the original drawings, and also the uniform courtesy of W. B. Saunders Company during the preparation of the book.

J. C. T.

DENVER, COLORADO.

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INTRODUCTION

USE OF THE MICROSCOPE

THERE is probably no laboratory instrument whose usefulness depends so much upon proper manipulation as the microscope, and none is so frequently misused by beginners. Some suggestions as to its proper use are, therefore, given at this place. It is presumed that the reader is already familiar with its general construction (Fig. 1).

For those who wish to understand the principles of the microscope and its manipulation—and best results are impossible without such an understanding—a careful study of some stan-
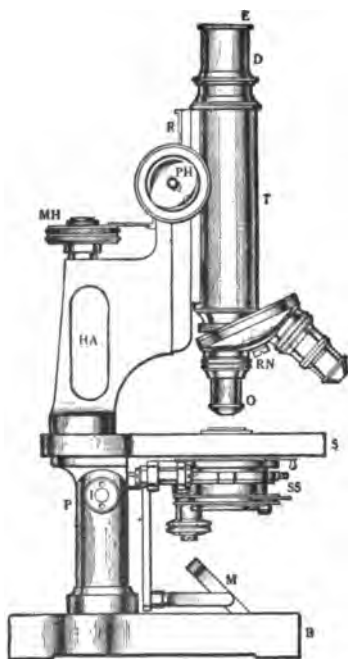
Fig. 1.—Handle-arm microscope: E, Eyepiece; D, draw-tube; T, body-tube; RN, revolving nose-piece; O, objective; PH, pinion head; MH, micrometer head; HA, handle-arm; SS, substage; S, stage; M, mirror; B, base; R, rack; P, pillar; I, inclination joint.

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dard work upon microscopy, such as those of Carpenter, Spitta, and Sir A. E. Wright, is earnestly recommended. It is also recommended that the beginner provide himself with some slides of diatoms, for example, *Pleurosigma angulatum*, *Surirella gemma*, and *Amphipleura pellucida*, costing fifty cents each. Faithful practice upon such test-objects, in the light of the principles of microscopy, will enable the student to reach, intelligently, an accuracy in manipulation to which the ordinary laboratory worker attains only slowly and by rule of thumb. He will soon find that the bringing of an object into accurate focus is by no means all of microscopy.

Illumination.—Good work cannot be done without proper illumination. It is difficult to lay too much stress upon this point.

The light which is generally recommended as best is that from a white cloud, the microscope being placed by preference at a north window, to avoid direct sunlight. Such light is satisfactory for all ordinary work. Artificial light is, however, imperative for those who must work at night, and is a great convenience at all times. Properly regulated artificial light, moreover, offers decided advantages over daylight for critical work. Almost any strong light which is diffused through a frosted globe will give fair results. The inverted Welsbach light with such a globe is excellent. The following plan is much used abroad, and gives results equal to the best daylight: A Welsbach lamp or strong electric light is used, and a glass globe—a six-inch round-bottom flask answers admirably—is placed between it and the microscope, to act as a condenser (Fig. 2).

The flask should be at a distance equal to its diameter from both the light and the mirror of the microscope. In order to filter out the yellow rays the flask is filled with water to which have been added a few crystals of copper sulphate and a little ammonia.

For critical work, the method suggested by Sir A. E. Wright is to be preferred. He has shown that fog is dispelled and definition is improved if the size of the light source is so regulated that its image, thrown upon
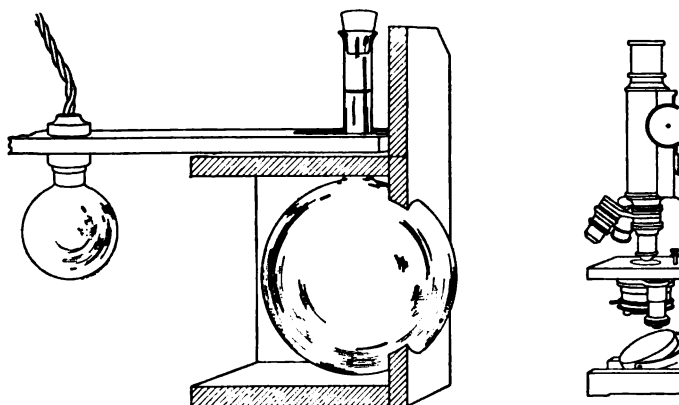
Fig. 2.—Illumination with water-bottle condenser.

the slide by the condenser, coincides with the real field of the objective. Upon this principle a very neat and satisfactory microscope lamp, shown in Fig. 3, has been designed by B. H. Matthews. It is fitted with iris-diaphragm, condensing lens, small electric light, and reflector, and has a slot in which a ray filter or ground-glass disc may be inserted.

Illumination may be either *central* or *oblique*. Central illumination is to be used for all routine work. To ob-

tain this, the mirror should be so adjusted that the light from the source selected is reflected directly up the tube of the microscope. This is easily done by removing the eye-piece and looking down the tube while adjusting the mirror. The eye-piece is then replaced, and the light reduced as much as desired by means of the diaphragm. With daylight, it is best to use the plane mirror; with artificial light, the concave mirror.
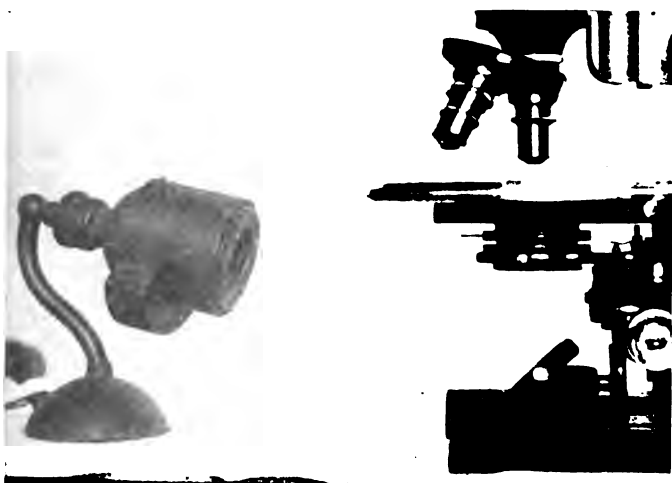
Fig. 3.—Matthews' microscope lamp with iris-diaphragm.

Oblique illumination is to be used only to bring out certain structures more clearly after viewing them by central light: as, for example, to show the edges of a hyaline cast by throwing one of its sides into shadow. Oblique illumination is obtained in the more simple instruments by swinging the mirror to one side, so that the light enters the microscope obliquely. The more complicated instruments obtain it by means of a rack

and pinion, which moves the diaphragm laterally. Beginners frequently use oblique illumination without recognizing it, and are thereby much confused. If the light be oblique, an object in the center of the field will appear to move from side to side when the fine adjustment is turned back and forth.

The amount of light is even more important than its direction. It is regulated by the diaphragm. *It is always best to use the least light that will show the object*

<center>*a* *b*</center>

Fig. 4.—*a*, Hyaline casts, one containing renal cells; properly subdued illumination; *b*, same as *a*; strong illumination. The casts are lost in the glare, and only the renal cells are seen. (From Greene's "Medical Diagnosis").

well. Unstained objects require very subdued light. Beginners constantly use it too strong. Strong light will often render semitransparent structures, as hyaline casts, entirely invisible (Fig. 4). Stained objects, especially bacteria, require much greater light.

Dark Ground Illumination.—This consists in cutting out the central rays of light and directing the peripheral rays against the object from the side. Only those rays which strike the object and are reflected pass into the objective. The object is bright upon a black back-

ground. By means of this form of illumination very
minute structures can be seen, just as particles of dust
in the atmosphere become visible when a ray of sunlight
enters a darkened room.

Dark ground illumination for low-power work can be
obtained by means of the ring stops with central discs
which accompany most microscopes when purchased.
The stop is placed in a special ring beneath the con-
denser. By varying the size of the central disc good
results can be had with the lower power dry lenses.

For oil-immersion work a special condenser is neces-
sary. With some makes it is placed upon the stage of
the microscope; with others it is substituted for the
regular condenser. It requires an intense light, like
direct sunlight or the Liliput arc-light.

The chief use of dark ground illumination in clinical
work is for demonstration of *Treponema pallidum* in
fresh material (Fig. 158).

The Condenser.—For the work of the clinical labora-
tory a substage condenser is a necessity. Its purpose
is to condense the light upon the object to be examined.
For critical work the light must be focused on the object
by raising or lowering the condenser by means of the
screw provided for the purpose. The image of the light
source will then appear in the plane of the object. This
is best seen by using a low-power objective and ocular.
Should the image of the window-frame or other nearby
object appear in the field and prove annoying, the con-
denser may be raised or lowered a little. It is often
advised to remove the condenser for certain kinds of
work, but this is not necessary and is seldom desirable.

It is very important that the condenser be accurately

centered, and most high-grade instruments have centering screws by which it can be adjusted at any time. The simplest way to recognize whether the condenser is centered is to close the diaphragm beneath it to as small an opening as possible, then remove the eye-piece and look down the tube. If the diaphragm opening does not appear in the center of the field, the condenser is out of center.

The use of the condenser is further discussed in the following section.

Objectives and Eye-pieces.—Unfortunately, different makers use different systems of designating their lenses. The best system, and the one chiefly used in this country, is to designate objectives by their focal lengths in millimeters, and eye-pieces by their magnifying power, indicated by an " ×." Most foreign makers use this system for their high-grade lenses, but still cling to arbitrary letters or numbers for their ordinary output.

Objectives are of two classes—achromatic and apochromatic. Those in general use are of the achromatic type, and they fulfil all requirements for ordinary work. Apochromatic objectives are more highly corrected for chromatic and spheric aberration, and represent the highest type of microscope lenses produced. They are very desirable for photomicrographic and research work, but for routine laboratory work do not offer advantages commensurate with their great cost. They require the use of special " compensating " eye-pieces.

The " working distance " of an objective should not be confused with its focal distance. The former term refers to the distance between the front lens of the objective, when it is in focus, and the cover-glass. It is

always less than the focal distance, since the " focal point " lies somewhere within the objective; and it varies considerably with different makes. Long working distance is a very desirable feature.

Objectives are " corrected " for use under certain fixed conditions, and *they will give the best results only when used under the conditions for which corrected.* The most important corrections are: (*a*) For tube-length; (*b*) for thickness of cover-glass; and (*c*) for the medium between objective and cover-glass.

(*a*) The tube-length with which an objective is to be used is usually engraved upon it—in most cases it is 160 mm. The draw-tube of the microscope should be pulled out until the proper length is obtained, as indicated by the graduations on its side. When a nose-piece is used, it adds about 15 mm. to the tube-length, and the draw-tube must be pushed in for that distance.

(*b*) The average No. 2 cover-glass is about the thickness for which most objectives are corrected—usually 0.17 or 0.18 mm. Very low powers and oil-immersion objectives do not require any cover-glass. A cover should always be used with high dry lenses, but its exact thickness is more important in theory than in practice. Many immersion objectives have such short working distance that only very thin covers can be used.

(*c*) The correction for the medium between objective and cover-glass is very important. This medium may be either air or some fluid, and the objective is hence either a " dry " or an " immersion " objective. The immersion fluid generally used is cedar oil, which gives great optical advantages because its index of refraction is the same as that of crown glass. It is obvious that only objectives

with very short working distance, as the 2 mm., can be used with an immersion fluid.

To use an oil-immersion objective a drop of the cedar oil which is prepared for the purpose should be placed upon the cover, and the objective lowered into it and then brought to a focus in the usual way.

Bubbles in the oil are a frequent source of trouble, and should always be looked for when an immersion objective does poor work. They are readily seen by removing the eye-piece and looking down the tube. Immediately after use the oil should be removed with lens-paper or a soft linen handkerchief.

A useful " pointer " can be made by placing a straight piece of a hair across the opening of the diaphragm of the eye-piece, cementing one end with a tiny drop of balsam, and cutting the hair in two in the middle. When the eye-piece is in place, the hair appears as a black line extending from the periphery to the center of the microscopic field.

Numeric Aperture.—This expression, usually written N. A., indicates the amount of light which enters an objective from a point in the microscopic field. In optical language, N. A. is the sine of one-half the angle of aperture multiplied by the index of refraction of the medium between the cover and the front lens. Numeric aperture is extremely important, because upon it depends *resolving power*, which is the most important property of an objective.[1]

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[1] Resolving power really depends upon two factors, the N. A. and the wave length of light, but the latter can be ignored in practice. The great resolving power of the ultra-microscope depends upon its use of light of short wave length.

Resolving power is the ability to separate minute details of structure. For example, the dark portions of a good half-tone picture appear gray or black to the unaided eye, but a lens easily resolves this apparently uniform surface into a series of separate dots. Resolving power does not depend upon magnification. The fine lines and dots upon certain diatoms may be brought out clearly and crisply (*i. e.*, they are resolved) by an objective of high numeric aperture, whereas with an objective of lower numeric aperture, but greater magnifying power, the same diatom may appear to have a smooth surface, with no markings at all, no matter how greatly it is magnified. Knowing the N. A., it is possible to calculate how closely lines and dots may lie and still be resolved by a given objective. To state the numeric aperture, therefore, is to tell what the objective can accomplish; provided, of course, that spheric and chromatic aberrations are satisfactorily corrected. An objective's N. A. is usually engraved upon the mounting.

It is an important fact, and one almost universally overlooked by practical microscopists, that the proportion of the numeric aperture of an objective which is *utilized* depends upon the aperture of the cone of light delivered by the condenser. In practice, the numeric aperture of an objective is reduced nearly to that of the condenser (which is indicated by lower-case letters, n. a.).[1] The condenser should, therefore, have a numeric aperture at least equal to that of the objective with which it is to be used. Lowering the condenser

[1] The N. A. of the objective is not reduced wholly to that of the condenser, because, owing to diffraction phenomena, a small part of the unilluminated portion of the black lens is utilized.

below its focal distance and closing the diaphragm beneath it have the effect of reducing its working aperture. A condenser, whatever its numeric aperture, cannot deliver through the air a cone of light of greater n. a. than 1. It follows, therefore, that the proper adjustment of the substage condenser is a matter of great importance when using objectives of high N. A., and that, to gain the full benefit of the resolving power of such objectives, the condenser must be focused on the object under examination, it must be oiled to the under surface of the slide in the same way as the immersion objective is oiled to the cover-glass, and the substage diaphragm must be wide open. The last condition introduces a difficulty in that colorless structures will appear "fogged" in a glare of light (Fig. 4). Wright suggests that the size of the light source be so regulated by a diaphragm that its image, thrown on the slide by the condenser, coincides with the real field of the objective. and maintains that in this way it is possible to reduce the glare of light and to dispel the fog without closing the diaphragm of the condenser.

One can easily determine how much of the aperture of an objective is in use by removing the eye-piece, looking down the tube, and observing what proportion of the back lens of the objective is illuminated. The relation of the illuminated central portion to the unilluminated peripheral zone indicates the proportion of the numeric aperture in use. The effect of raising and lowering the condenser and of oiling it to the slide can thus be easily seen.

Magnification.—The degree of magnification should always be expressed in *diameters*, not *times*, which is a

misleading term. The former refers to increase of *diameter;* the latter, to increase of *area.* The comparatively low magnification of 100 diameters is the same as the apparently enormous magnification of 10,000 times.

The magnifying power of a lens is obtained by dividing 250 mm., or 10 inches (the distance of normal vision), by the focal length of the lens. The focal length of an objective is approximately twice the diameter of the front lens. Thus, the 2 mm. objective gives a magnification of 125 diameters; the 25 mm. eye-piece gives a magnification of 10 diameters, and is usually designated as a 10× eye-piece. When an objective and eye-piece are used together, the total magnification is the product of the two. In the case just cited the total magnification would be 1250 diameters. In practice, magnification can be increased in one of three ways:

(*a*) *Drawing out the tube.* Since the increased tube-length interferes with spheric correction, it should be used only with the knowledge that an imperfect image will result.

(*b*) *Using a higher power objective.* As a rule, this is the best way, because resolving power is also increased; but it is often undesirable because of the shorter working distance, and because the higher objective often gives greater magnification than is desired, or cuts down the size of the real field to too great an extent.

(*c*) *Using a shorter eye-piece.* This is the simplest method. It has, however, certain limitations. When too high an eye-piece is used, there results a hazy image in which no structural detail is seen clearly. This is called " empty magnification," and depends upon the fact that the objective has not sufficient resolving power

to support the high magnification.   The extent to which magnification can be satisfactorily increased by eye-piecing depends wholly upon the resolving power of the objective, and consequently upon the N. A.   The greatest total or combined magnification which will give an *absolutely* crisp picture is found by multiplying the N. A. of an objective by 400.   The greatest magnification which can be used at all satisfactorily is 1000 times the N. A.   For example: The ordinary 2 mm. objective has a N. A. of 1.30; the greatest magnification which will give an absolutely sharp picture is 520 diameters, which is obtained approximately by using a 4× eye-piece. Higher eye-pieces can be used, up to a total magnification of 1300 diameters (10× eye-piece), beyond which the image becomes wholly unsatisfactory.

Focusing.—It is always best to " focus up," which saves annoyance and probable damage to slides and objectives.   This is accomplished by bringing the objective nearer the slide than the proper focus, and then, with the eye at the eye-piece, turning the tube up until the object is clearly seen. *The fine adjustment should be used only to get an exact focus with the higher power objectives after the instrument is in approximate focus.* It should not be turned more than one revolution.

There will be less fatigue to the eyes if both are kept open while using the microscope, and if no effort is made to see objects which are out of distinct focus.   Fine focusing should be done with the fine adjustment, not with the eye.   An experienced microscopist keeps his fingers almost constantly upon one or other of the focusing adjustments.   Greater skill in recognizing objects will be acquired if the same eye be always used.   To be

seen most clearly, an object should be brought to the center of the field.

Care of the Microscope.—The microscope is a delicate instrument and should be handled accordingly. It is so heavy that one is apt to forget that parts of it are fragile. It seems unnecessary to say that when there is unusual resistance to any manipulation, force should never be used to overcome it until its cause has first been sought; and yet it is no uncommon thing to see students, and even graduates, push a high-power objective against a microscopic preparation with such force as to break not only the cover-glass, but even a heavy slide.

It is most convenient to carry a microscope with the fingers grasping the pillar and the arm which holds the tube; but since this throws a strain upon the fine adjustment, it is safer to carry it by the base. In the more recent instruments a convenient handle-arm is provided. To bend the instrument at the joint, the force should be applied to the pillar and never to the tube or the stage.

Lens surfaces which have been exposed to dust only should be cleaned with a camel's-hair brush. Those which are exposed to finger-marks should be cleaned with lens paper, or a soft linen handkerchief wet with saliva. Particles of dirt which are seen in the field are upon the slide, the eye-piece, or the condenser. Their location can be determined by moving the slide, rotating the eye-piece, and lowering the condenser. When the image is hazy, the objective probably needs cleaning; or in case of an oil-immersion lens, there may be bubbles in the oil.

Oil and balsam which have dried upon the lenses and resist saliva may be removed with alcohol or xylol; but

these solvents must be used sparingly and carefully, as there is danger of softening the cement.   Care must be taken not to get any alcohol upon the brass parts, as it will remove the lacquer.   Balsam and dried oil are best removed from the brass parts with xylol.

Choice of a Microscope.—It is poor economy to buy a cheap instrument.

For the work of a clinical laboratory the microscope should preferably be of the new handle-arm type, and should have a large stage.   It should be provided with a substage condenser (preferably of 1.40 n. a.), three or more objectives, and two or more eye-pieces.

The most generally useful objectives are: 16 mm., 4 mm., and 2 mm. oil immersion.   The 4 mm. objective may be obtained with N. A. of 0.65 or 0.85.   If it is to be used for blood-counting, the former is preferable, since its working distance is sufficient to take the thick cover of the Thoma-Zeiss instrument.   For coarse objects a 32 mm. objective is very desirable.   The eye-pieces most frequently used are 4× and 8×.   A very low power (2×) and a very high (18×) will sometimes be found useful.   The micrometer eye-piece is almost a necessity. A mechanical stage, preferably of the attachable type, is almost indispensable for blood and certain other work.

A first-class microscope, of either American or foreign make, equipped as just described, will cost in the neighborhood of a hundred dollars, exclusive of the mechanical stage.

Measurement of Microscopic Objects.—Of the several methods, the most convenient is the use of a micrometer eye-piece.   In its simplest form this is similar to an ordinary eye-piece, but has within it a glass disc upon

which is ruled a graduated scale. When this eye-piece is placed in the tube of the microscope, the ruled lines appear in the microscopic field, and the size of an object is readily determined in *terms of the divisions of this scale*. The value of these divisions in inches or millimeters manifestly varies with different magnifications. Their value must, therefore, be determined separately for each objective. This is accomplished through use of a stage micrometer—a glass slide with carefully ruled scale divided into hundredths and thousandths of an inch, or into subdivisions of a millimeter. The stage micrometer is placed upon the stage of the microscope and brought into focus. From the number of divisions of the eye-piece scale corresponding to each division of the stage micrometer the value of the former in fractions of an inch or millimeter is easily calculated. The counting slide of the Thoma-Zeiss hemocytometer will answer in place of a stage micrometer, the lines which form the sides of the small squares being one-twentieth of a millimeter apart. Any eye-piece can be converted into a micrometer eye-piece by placing a micrometer disc—a small circular glass plate with ruled scale—ruled side down upon its diaphragm.

The principal microscopic objects which are measured clinically are animal parasites and their ova and abnormal blood-corpuscles. The metric system is used almost exclusively. For very small objects 0.001 mm. has been adopted as the unit of measurement, under the name *micron*. It is represented by the Greek letter μ. For larger objects, where exact measurement is not essential, the diameter of a red blood-corpuscle (7 to 8 μ) is sometimes taken as a unit.

Tuttle has suggested that in feces and other examinations a little lycopodium powder be mixed with the material. The granules are of uniform size—30 μ in diameter—and are easily recognized (Fig. 5). They furnish a useful standard with which the size of other structures can be compared.
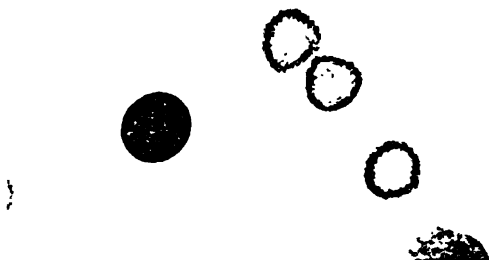
Fig. 5.—Egg of Tænia saginata. Lycopodium granules used as micrometer ($\times$ 250) (photograph by the author).

Photomicrography.—Very satisfactory pictures of microscopic structures can be made by any one with simple apparatus.

Any camera with focusing screen or a Kodak with plate attachment may be used. It is best, but not necessary, to remove the photographic lens. The camera is placed with the lens (or lens-opening, if the lens has been removed) looking into the eye-piece of the microscope, which may be in either the vertical or the horizontal position. One can easily rig up a standard to which the camera can be attached in the proper position by means of a tripod screw. A light-tight connection can be made

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of a cylinder of paper or a cloth sleeve with draw-strings. The image will be thrown upon the ground-glass focusing screen, and is focused by means of the fine adjustment of the microscope. The degree of magnification is ascertained by placing the ruled plate of the blood-counting instrument upon the microscope and measuring the image on the screen. The desired magnification is obtained by changing objectives or eye-pieces or lengthening the camera-draw.

Focusing is comparatively easy with low powers, but when using an oil-immersion objective, it is a difficult problem unless the source of light be very brilliant. If one always uses the same length of camera and microscope tube, a good plan is as follows: Ascertain by trial with a strong light how far the fine adjustment screw must be turned from the correct eye focus to bring the image into sharp focus upon the ground-glass screen. At any future time one has only to focus accurately with the eye, bring the camera into position, and turn the fine adjustment the required distance to right or left.

The light should be as intense as possible, in order to shorten exposure, but any light that is satisfactory for ordinary microscopic work will answer. It is nearly always necessary to insert a color screen between the light and the microscope. Pieces of colored window-glass are useful for this purpose. The screen should have a color complementary to that which it is desired to bring out strongly in the photograph: for blue structures, a yellow screen; for red structures, a green screen. For the average stained preparation, a picric-acid yellow or a yellow green will be found satisfactory.

Very fair pictures can be made on Kodak film, but

orthochromatic plates (of which Cramer's " Iso " and Seed's " Ortho " are examples) give much better results. The length of exposure depends upon so many factors that it can be determined only by trial. It will probably vary from a few seconds to fifteen minutes. Plates are developed in the usual way,—the tank method yielding most uniform and satisfactory results,—but in order to secure all the contrast possible, they should be considerably overdeveloped.
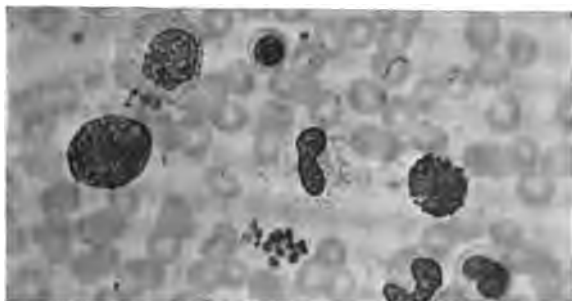
Fig. 6.—Leukemic blood (about × 650). Photograph taken with a kodak, as described in the text.

The photograph from which Fig. 6 was made was taken with a Kodak and plate attachment on an " Iso " plate, the source of light being the electric lamp and condensing lens illustrated in Fig. 2. It was focused by the method described above. The screen was a picric-acid stained photographic plate. Exposure, three and a half minutes. The picture loses considerable detail in reproduction.

CHAPTER I

THE SPUTUM

Preliminary Considerations.—Before beginning the study of the sputum, the student will do well to familiarize himself with the structures which may be present in the normal mouth, and which frequently appear in the sputum as contaminations. Nasal mucus and material obtained by scraping the tongue and about the teeth should be studied as described for unstained sputum. A drop of Lugol's solution should then be placed at the edge of the cover, and, as it runs under, the effect upon different structures noted. Another portion should be spread upon slides or covers and stained by some simple stain and by Gram's method. The structures likely to be encountered are epithelial cells of columnar and squamous types, leukocytes, food-particles, *Leptothrix buccalis*, and great numbers of saprophytic bacteria, frequently including spirochetes. These structures are described later.

The morning sputum or the whole amount for twenty-four hours should be collected for examination. In beginning tuberculosis tubercle bacilli can often be found in that first coughed up in the morning when they cannot be detected at any other time of day. Sometimes, in these early cases, there are only a few mucopurulent flakes which contain the bacilli, or only a small purulent mass every few days, and these may easily be overlooked.

Patients should be instructed to rinse the mouth well in order to avoid contamination with food-particles which may prove confusing in the examination, and to make sure that the sputum comes from the lungs or bronchi and not from the nose and nasopharynx. Many persons find it difficult to distinguish between the two. It is always desirable that the material be raised with a distinct expulsive cough, but this is not always possible. Material from the upper air-passages can usually be identified from the large proportion of mucus and the character of the epithelial cells.

As a receptacle for the sputum, a clean, wide-mouthed bottle with tightly fitting cork may be used. The patient must be particularly cautioned against smearing any of it upon the outside of the bottle. This is probably the chief source of danger to those who examine sputum. Disinfectants should not be added. They so alter the character of the sputum as to render it unfit for satisfactory examination.

When the examination is begun, the material should be spread out in a thin layer in a Petri dish, or between two small plates of glass, like photographic plates. It may then be examined with the naked eye—best over a black background—or with a low power of the microscope. The portions most suitable for further examination may thus be easily selected. *This macroscopic examination should never be omitted.*

After an examination the sputum must be destroyed by heat or chemicals, and everything which has come in contact with it must be sterilized. The utmost care must be taken not to allow any of it to dry and become disseminated through the air. It is a good plan to con-

duct the examination upon a large newspaper, which can then be burned. Contamination of the work table is thus avoided. If this is not feasible, the table should be washed off with 10 per cent. lysol solution, and allowed to dry slowly, as soon as the sputum work is finished.

Examination of the sputum is most conveniently considered under four heads: I. Physical examination. II. Microscopic examination. III. Chemic examination. IV. Characteristics of the sputum in various diseases.

I. PHYSICAL EXAMINATION

1. Quantity.—The quantity expectorated in twenty-four hours varies greatly. It may be so slight as to be overlooked entirely in beginning tuberculosis. It is usually small in acute bronchitis and lobar pneumonia. It may be very large—sometimes as much as 1000 c.c.— in advanced tuberculosis with large cavities, edema of the lung, bronchiectasis, and following rupture of an abscess or empyema. It is desirable to obtain a general idea of the quantity, but accurate measurement is unnecessary.

2. Color.—Since the sputum ordinarily consists of varying proportions of mucus and pus, it may vary from a colorless, translucent mucus to an opaque, whitish or yellow, purulent mass. A yellowish green is frequently seen in advanced phthisis and chronic bronchitis. In jaundice, in caseous pneumonia, and in slowly resolving lobar pneumonia it may assume a bright green color, due to bile or altered blood-pigment.

A red color usually indicates the presence of blood. Bright red blood, most commonly in streaks, is strongly suggestive of phthisis. It may be noted very early in the disease. A rusty red sputum is the rule in croupous

pneumonia, and was at one time considered pathogno-
monic of the disease. " Prune-juice " sputum is said to
be characteristic of " drunkard's pneumonia." It at
least indicates a dangerous type of the disease. A
brown color, due to altered blood-pigment, follows
hemorrhages from the lungs, and is present, to greater or
less degree, in chronic passive congestion of the lung,
which is most frequently due to a heart lesion.

Gray or black sputum is observed among those who
work much in coal-dust, and is occasionally seen in
smokers who are accustomed to " inhale."

3. Consistence.—According to their consistence, sputa
are usually classified as serous, mucoid, purulent, sero-
purulent, mucopurulent, etc., which names explain them-
selves. As a rule, the more mucus and the less pus and
serum a sputum contains, the more tenacious it is.

The rusty sputum of croupous pneumonia is extremely
tenacious, so that the vessel in which it is contained may
be inverted without spilling it. The same is true of the
almost purely mucoid sputum (" sputum crudum ") of
beginning acute bronchitis, and of that which follows
an attack of asthma. A purely serous sputum, usually
slightly blood tinged, is fairly characteristic of edema of
the lungs.

4. Dittrich's Plugs.—While these bodies sometimes
appear in the sputum, they are more frequently ex-
pectorated alone. They are caseous masses, usually
about the size of a pin-head, but sometimes reaching
that of a bean. The smaller ones are yellow, the larger
ones gray. When crushed, they emit a foul odor.
Microscopically, they consist of granular débris, fat-glob-
ules, fatty acid crystals, and bacteria. They are formed

in the bronchi, and are sometimes expectorated by healthy persons, but are more frequent in putrid bronchitis and bronchiectasis. The laity commonly regard them as evidence of tuberculosis. The similar caseous masses which are formed in the crypts of the tonsils are sometimes also included under this name.

II. MICROSCOPIC EXAMINATION

The portions most likely to contain structures of interest should be very carefully selected, as already described. *The few minutes spent in this preliminary examination will sometimes save hours of work later.* Opaque, white or yellow particles are most frequently bits of food, but may be cheesy masses from the tonsils; small cheesy nodules, derived from tuberculous cavities and containing many tubercle bacilli and elastic fibers; Curschmann's spirals, or small fibrinous casts, coiled into little balls; or shreds of mucus with great numbers of entangled pus-corpuscles. The food-particles most apt to cause confusion are bits of bread, which can be recognized by the blue color which they assume when touched with iodin solution.

Some structures are best identified without staining; others require that the sputum be stained.

A. UNSTAINED SPUTUM

A careful study of the unstained sputum should be included in every routine examination. It best reveals certain structures which are seen imperfectly or not at all in stained preparations. It gives a general idea of the other structures which are present, such as pus-

corpuscles, eosinophiles, epithelial cells, and blood, and thus suggests appropriate stains to be used later.

The particle selected for examination should be transferred to a clean slide, covered with a clean cover-glass, and examined with the 16 mm. objective, followed by the 4 mm. It is convenient to handle the bits of sputum with a wooden tooth-pick or with a wooden cotton-applicator, which may be burned when done with. The platinum wire used in bacteriologic work is less satisfactory because not usually stiff enough.

The more important structures to be seen in unstained sputum are: elastic fibers, Curschmann's spirals, Charcot-Leyden crystals, fibrinous casts, the ray fungus of actinomycosis, and molds. Pigmented cells, especially the so-called " heart-failure cells " (p. 62), are also best studied without staining (Plate II, Fig. 1).

1. Elastic Fibers.—These are the elastic fibers of the pulmonary substance (Fig. 7). When found in the sputum, they always indicate destructive disease of the lung, provided they do not come from the food, which is a not infrequent source. They are found most commonly in phthisis; rarely in other diseases. Advanced cases of tuberculosis often show great numbers, and, rarely, they may be found in early tuberculosis when the bacilli cannot be detected. In gangrene of the lung, contrary to the older teaching, elastic tissue is probably always present in the sputum, usually in large fragments.

The fibers should be searched for with a 16 mm. objective, although a higher power is needed to identify them with certainty. Under the 4 mm. they appear as slender, highly refractive fibers with double contour, and often curled or split ends. Frequently they are found

in alveolar arrangement, retaining the original outline of the alveoli of the lung (Fig. 7, *b*). This arrangement is positive proof of their origin in the lung. *Leptothrix buccalis*, which is a normal inhabitant of the mouth, may easily be mistaken for elastic tissue. It can be distinguished by running a little iodin solution under the cover-glass (see p. 56).
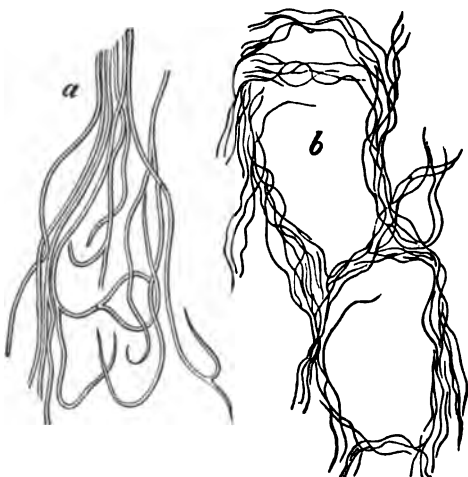
Fig. 7.—Elastic fibers from the sputum: *a*, Highly magnified; *b*, alveolar arrangement, less highly magnified (after Bizzozero).

Fatty-acid crystals, which are often present in Dittrich's plugs and in sputum which has lain in the body for some time, also simulate elastic tissue when very long, but they are more like stiff, straight or curved needles than wavy threads. They show varicosities when the cover-glass is pressed upon. The structures which most frequently confuse the student are the cotton fibrils which are present as a contamination in most

sputa. These are usually coarser than elastic fibers, and flat, with one or two twists, and often have longitudinal striations and frayed-out ends.

To find elastic fibers when not abundant, boil the sputum with a 10 per cent. solution of caustic soda until it becomes fluid; add several times its bulk of water, and centrifugalize, or allow to stand for twenty-four hours in a conical glass. Examine the sediment microscopically. The fibers will be pale and swollen and, therefore, somewhat difficult to recognize. Too long boiling will destroy them entirely.

The above procedure, although widely recommended, will rarely or never be necessary if the sputum is carefully examined in a thin layer against a black background macroscopically and with a hand-lens, and if all suspicious portions are further studied with the microscope.

2. Curschmann's Spirals.—These peculiar structures are found most frequently in bronchial asthma, of which they are fairly characteristic. They may occasionally be met with in chronic bronchitis and other conditions. Their nature has not been definitely determined.

Macroscopically, they are whitish or yellow, twisted threads, frequently coiled into little balls (Fig. 8, I). Their length is rarely over half an inch, though it sometimes exceeds two inches. Under a 16 mm. objective they appear as mucous threads having a clear central fiber, about which are wound many fine fibrils (Fig. 8, II. and III.). Eosinophiles are usually present within them, and sometimes Charcot-Leyden crystals. Not infrequently the spirals are imperfectly formed, con-

sisting merely of twisted strands of mucus inclosing leukocytes. The central fiber is absent from these.

3. **Charcot-Leyden Crystals.**—Of the crystals which may be found in the sputum, the most interesting are the Charcot-Leyden crystals. They may be absent when the sputum is expectorated, and appear in large numbers after it has stood for some time. They are rarely found
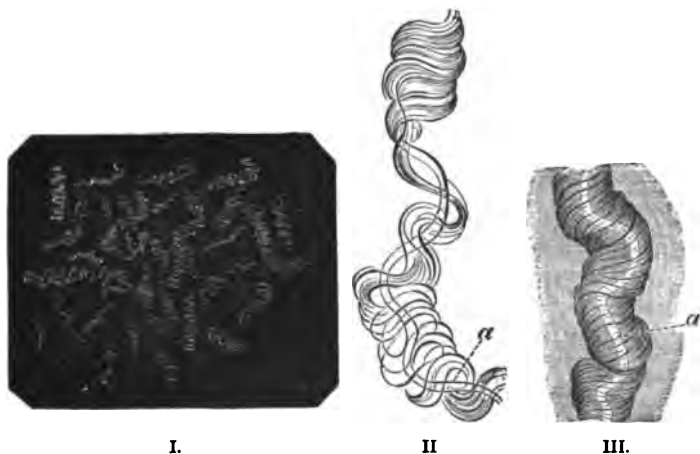
Fig. 8.—Curschmann's spirals: I., Natural size; II. and III., enlarged: a, central fiber (after Curschmann).

except in cases of bronchial asthma, and were at one time thought to be the cause of the disease. They frequently adhere to Curschmann spirals. Their exact nature is unknown. Their formation seems to be in some way connected with the presence of eosinophilic cells. Outside of the sputum they are found in the feces in association with animal parasites, and in the coagulated blood in leukemia.

They are colorless, pointed, often needle-like, octa-hedral crystals (Fig. 9). Their size varies greatly, the average length being about three or four times the diameter of a red blood-corpuscle.

Fig. 9.—Charcot-Leyden crystals (after Riegel).

Other crystals—hematoidin, cholesterin, and, most frequently, fatty-acid needles (see Fig. 36)—are common in sputum which has remained in the body for a consider-able time, as in abscess of the lung and bronchiectasis.

4. Fibrinous Casts.—These are casts of the bronchi, frequently, but not always, composed of fibrin. In color they are usually white or grayish, but may be reddish or brown, from the presence of blood-pigment. Their size varies with that of the bronchi in which they are formed. They may, rarely, be fifteen or more centimeters in length. When large, they can be recog-

nized with the naked eye by floating them out in water over a black surface; when small, a low power of the microscope must be used. Their branching, tree-like structure (Fig. 10) is usually sufficient to identify them.

Fibrinous casts are characteristic of fibrinous bronchitis, but may also be found in diphtheria of the smaller bronchi. Very small casts are often seen in croupous pneumonia.

Fig. 10.—Fibrinous bronchial cast (Sahli).

5. Actinomyces Bovis (Ray-fungus).—In the sputum of pulmonary actinomycosis and in the pus from actinomycotic lesions elsewhere small, yellowish, " sulphur " granules can be detected with the unaided eye. Without a careful macroscopic examination they are almost certain to be overlooked. The fungus can be seen by crushing one of these granules between slide and cover, and examining with a low power. It consists of a net-

work of threads having a more or less radial arrangement, those at the periphery presenting club-shaped extremities (Fig. 11). It can be brought out more clearly by running a little solution of eosin in alcohol and glycerin under the cover. This organism, also called *Streptothrix actinomyces*, apparently stands midway between the bacteria and the molds. It stains by Gram's method.

Actinomycosis of the lung is rare. The clinical picture is that of tuberculosis.

Fig. 11.—Sputum from a case of actinomycosis; stained (Jakob).

6. Molds and Yeasts.—The hyphæ and spores of various molds are occasionally met with in the sputum. They are usually the result of contamination, and have little significance. The hyphæ are rods, usually jointed or branched (Fig. 62), and often arranged in a meshwork (mycelium); the spores are highly refractive spheres. Both stain well with the ordinary stains.

In the extremely rare condition of systemic blastomycosis the specific yeasts have been found in the sputum

in large numbers. It is advisable to add a little 10 per cent. caustic soda solution and examine unstained.

7. Animal Parasites.—These are extremely rare in the sputum in this country. A trichomonad, perhaps identical with *Trichomonas vaginalis*, has been seen in the sputum of putrid bronchitis and gangrene of the lung, but its causal relationship is doubtful. In Japan, infection with the lung flukeworm, *Paragonimus westermani*, is common, and the ova are found in the sputum. The lung is not an uncommon seat for echinococcus cysts, and hooklets and scolices may appear, as may also *Amœba histolytica*, when a hepatic abscess has ruptured into the lung. Ciliated body-cells, with cilia in active motion, are not infrequently seen, and may easily be mistaken for infusoria. All the above-mentioned parasites are described in Chapter VI.

B. Stained Sputum

Structures which are best seen in stained sputum are bacteria and cells.

A number of smears should be made upon slides or covers, dried in the air, and fixed in the flame, as described on the next page. Fixation will kill the bacteria when covers are used, and the smears may be kept indefinitely; but smears on slides are often not sterile, and should be handled accordingly. One of the smears should be stained with some simple stain, like Löffler's methylene-blue, which will give a good idea of the various cells and bacteria present. Special stains may then be applied, as indicated, but a routine examination should, in all cases, include a stain by the method for the tubercle bacillus and by Gram's method.

1. Bacteria.—Saprophytic bacteria from mouth contamination are frequently present in large numbers and will prove confusing to the inexperienced. The presence of squamous cells in their neighborhood will suggest their source. Among the pathogenic organisms which have clinical importance are: tubercle bacilli; staphylococci and streptococci; pneumococci; bacilli of Friedländer; influenza bacilli, and *Micrococcus catarrhalis.*

(1) **Tubercle Bacillus.**—The presence of the tubercle bacillus may be taken as positive evidence of the existence of tuberculosis somewhere along the respiratory tract, most likely in the lung. In laryngeal tuberculosis it is not easily found in the sputum, but can frequently be detected in swabs made directly from the larynx.

Recognition of the tubercle bacillus depends upon the fact that it stains with difficulty; but that when once stained, it retains the stain tenaciously, even when treated with a mineral acid, which quickly removes the stain from other bacteria. This " acid-fast " property is due to the presence of a waxy capsule. The most convenient method for general purposes is here given in detail:

Gabbet's Method.—(1) Spread suspicious particles thinly and evenly upon a slide or a cover-glass held in the grasp of cover-glass forceps. In general, slides are more satisfactory, but cover-glasses are easier to handle while staining. Do not grasp a cover too near the edge or the stain will not stay on it well. Tenacious sputum will spread better if gently warmed while spreading.

(2) Dry the film in the air.

4

(3) Fix in a flame; *i. e.*, pass the cover-glass rather slowly, with film side up, three times (a slide about twelve times) through the flame of a Bunsen burner or alcohol lamp low down in the flame. Take care not to scorch. Should the film be washed off during future manipulations, fixation has been insufficient.

(4) Apply as much carbolfuchsin as will stay on, and hold over a flame so that it will steam for three minutes or longer, replacing the stain as it evaporates. If the bacilli are well stained in this step, there will be little danger of decolorizing them later. Too great heat will interfere with the staining of some of the bacilli, probably by destroying the waxy envelop upon which the acid-fast property depends. It is better to stain at room temperature for twelve to twenty-four hours.

(5) Wash the film in water.

(6) Apply Gabbet's stain to the under side of the cover-glass to remove excess of carbolfuchsin, and then to the film side. Allow this to act for one-fourth to one-half minute.

(7) Wash in water.

(8) If, now, the thinner portions of the film are blue, proceed to the next step; if they are still red, repeat steps (6) and (7) until the red has disappeared. Too long application of Gabbet's stain will decolorize the tubercle bacilli.

(9) Place the preparation between layers of filter-paper and dry by rubbing with the fingers, as one would in blotting ink.

(10) Put a drop of Canada balsam upon a clean slide, place the cover-glass film side down upon it, and examine with an immersion objective. Cedar oil or water may be used in place of balsam for temporary preparations. Smears on slides may be examined directly with an oil-immersion lens, no cover being necessary.

Carbolfuchsin is prepared by mixing 10 c.c. of a saturated alcoholic solution of fuchsin with 90 c.c. of 5 per cent. aqueous solution of phenol.

Gabbet's stain consists of methylene-blue, 2 gm.; 25 per cent. sulphuric acid, 100 c.c.

Both stains can be purchased ready prepared.

Other Methods.—The objection is often raised that decolorization is masked by the blue in Gabbet's stain, but this will not make trouble if step 8 is carefully carried out. The **Ziehl-Neelsen method** is preferred by many: After the staining with carbolfuchsin the smear is washed in 5 per cent. nitric acid until decolorized, washed in water, stained lightly with Löffler's methylene-blue, again washed, and mounted.

Pappenheim's Method.—This is the same as Gabbet's method, except that Pappenheim's methylene-blue solution is substituted for Gabbet's solution. This consists of:

Corallin (rosolic acid) .... 1 gm.
Absolute alcohol...................... 100 c.c.
Saturate with methylene-blue and add 20 c.c. glycerin.

The method is very satisfactory for routine work. Decolorization of the tubercle bacillus is practically impossible: it retains its red color, even when soaked overnight in Pappenheim's solution. The stain was originally recommended as a means of differentiating the smegma bacillus, which is decolorized by it; but it is not to be absolutely relied upon for this purpose.

In films stained by these methods tubercle bacilli, if present, will be seen as slender red rods upon a blue background of mucus and cells (Plate II, Fig. 2). They average 3 to 4 μ in length—about one-half the diameter of a red blood-corpuscle. Beginners must be warned against mistaking the edges of cells, or particles which have retained the red stain, for bacilli. The appearance of the bacilli is almost always typical, and if there

seems room for doubt, the structure in question is probably not a tubercle bacillus. They may lie singly or in groups. They are very frequently bent and often have a beaded appearance. It is possible that the larger, beaded bacilli indicate a less active tuberculous process than do the smaller, uniformly stained ones. Sometimes they are present in great numbers—thousands in a field of the 2 mm. objective. Sometimes several cover-glasses must be examined to find a single bacillus. At times they are so few that none are found in stained smears, and special methods are required to detect them. The number may bear some relation to the severity of the disease, but this relation is by no means constant. The mucoid sputum from an incipient case sometimes contains great numbers, while sputum from large tuberculous cavities at times contains very few. Failure to find them is not conclusive, though their absence is much more significant when the sputum is purulent than when it is mucoid.

When they are not found in suspicious cases, one of the following methods should be tried:

(1) **Antiformin Method.**—This has lately come into use, and has superseded the older methods of concentration. The chief difficulty with the older methods, such as boiling with caustic soda, is that the bacilli are so injured in the process that they do not stain characteristically.

Antiformin is the patented name for a preparation consisting essentially of equal parts of a 15 per cent. solution of caustic soda and a 20 per cent. solution of sodium hypochlorite. It keeps fairly well. The sputum is thoroughly shaken in a corked bottle with one-fourth its volume of antiformin, and allowed to stand four to six hours in an incubator,

PLATE II

Fig. 1.—Heart-failure cells in sputum, containing blood-pigment, from a case of cardiac congestion of the lungs (Jakob).
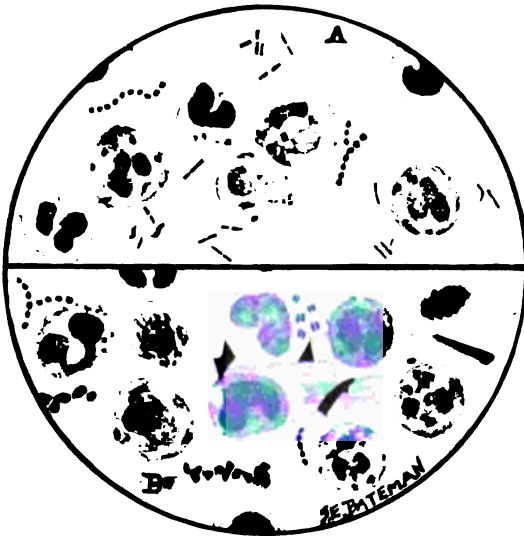

Fig. 2.—*A*, Sputum showing tubercle bacilli stained with carbolfuchsin and Gabbet's methylene-blue solution (obj. one-twelfth oil-immersion); *B*, sputum of anthracosis, showing particles of coal-dust stained with methylene-blue (obj. one-twelfth oil-immersion) (Boston).

or twenty-four hours at room-temperature.   The sputum will
be thoroughly liquefied. A centrifuge tubeful is thoroughly
centrifugalized, the supernatant fluid is poured off and
replaced with water, and centrifugalization is repeated.
This washing is repeated several times.   Some of the sedi-
ment is then spread upon slides (with a little egg-albumen
or some of the untreated sputum to cause it to adhere),
dried, fixed, and stained.   The tubercle bacilli are not killed,
but retain their form and staining properties unchanged.
Other bacteria and cells are destroyed.

   Since the bacilli remain alive, the utmost care must be
used in handling, and all tubes and glassware which have
come in contact with the liquefied sputum must be
sterilized.

   (2) **Animal Inoculation.**—Inoculation of guinea-pigs is
the court of last appeal in detection of tubercle bacilli.   The
method is described on p. 375·

There are a number of bacilli, called *acid-fast bacilli*,
which stain in the same way as the tubercle bacillus.
They stain with difficulty, and when once stained, retain
the color even when treated with a mineral acid; but,
unlike the tubercle bacillus, most of them can be decolor-
ized with alcohol.   Of these, the smegma bacillus is the
only one likely ever to cause confusion.   It, or a similar
bacillus, is sometimes found in the sputum of gangrene
of the lung.   It occurs normally about the glans penis
and the clitoris, and is often present in the urine and in
the wax of the ear.   The method of distinguishing it
from the tubercle bacillus is given later (p. 168).

   Other bacteria than the acid-fast group are stained
blue by Gabbet's method.   Those most commonly found
are staphylococci, streptococci, and pneumococci.   Their

presence in company with the tubercle bacillus constitutes *mixed infection*, which is much more serious than single infection by the tubercle bacillus. It is to be remembered, however, that a few of these bacteria may reach the sputum from the upper air-passages. Clinically, mixed infection is evidenced by fever.

(2) **Staphylococcus and Streptococcus** (p. 368).—One or both of these organisms is commonly present in company with the tubercle bacillus in the sputum of advanced phthisis (Plate II, Fig. 2). They are often found in bronchitis, catarrhal pneumonia, and many other conditions.

(3) **Pneumococcus (Diplococcus of Fränkel).**—The pneumococcus is the causative agent in nearly all cases of croupous pneumonia, and is commonly found in large numbers in the rusty sputum of this disease. It is sometimes met with in the sputum of catarrhal pneumonia, bronchitis, and tuberculosis. It is also an important factor in the causation of pleurisy, meningitis, otitis media, and other inflammations. It has been found in the saliva in health. Pneumococci are about the size of streptococci. They are ovoid in shape, and lie in pairs, end to end, often forming short chains. Each is surrounded by a gelatinous capsule, which is its distinctive feature (Fig. 12). Diplococci without capsules are common in the sputum, but have no special significance.

The pneumococcus is closely related to the streptococcus, and it is sometimes extremely difficult to differentiate them even by culture methods (for which see p. 368). The morphology of the pneumococcus, the fact that it is Gram-positive, and the presence of a capsule are, however, generally sufficient for its recognition

in smears from sputum or pus. The capsule is often seen as a halo around pairs of cocci in smears stained by the ordinary methods, particularly Gram's method, but to show it well special methods are required. There are numerous special methods of staining capsules which are applicable to other encapsulated bacteria, as well as to the pneumococcus, but few of them are satisfactory. Buerger's method can be recommended. It is especially useful with cultures upon serum media,
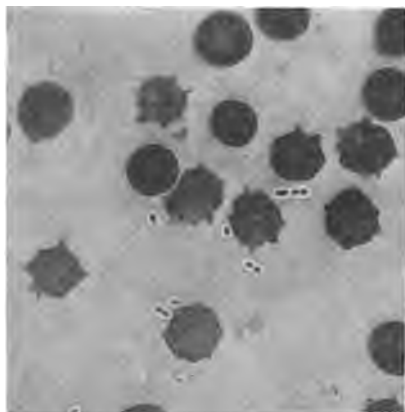

Fig. 12.—Diplococcus pneumoniæ in the blood (× 1000) (Fränkel and Pfeiffer).

but is applicable also to the sputum. Smith's new method is easier of application, and apparently gives uniformly good results. The India-ink method described for the organism of syphilis is likewise said to show capsules satisfactorily. The sputum should be fresh— not more than three or four hours old.

Buerger's Method for Capsules.—(1) Mix a few drops each of the sputum and blood-serum or egg-albumen solu-

tion (egg-albumen, distilled water, equal parts; shake and filter through cotton). Blood-serum can be obtained as described for the Widal test, p. 258. Make thin smears from the mixture, and just as the edges begin to dry, cover with Müller's fluid (potassium dichromate, 2.5 gm.; sodium sulphate, 1.0 gm.; water, 100 c.c.) saturated with mercuric chlorid (ordinarily about 5 per cent.). Gently warm over a flame for about three seconds.

(2) Rinse very quickly in water.

(3) Flush once with alcohol.

(4) Apply tincture of iodin for one to two minutes.

(5) Thoroughly wash off the iodin with alcohol and dry in the air.

(6) Stain about three seconds with weak anilin-gentian-violet freshly made up as follows: Anilin oil, 10; water, 100; shake; filter; and add 5 c.c. of a saturated alcoholic solution of gentian violet.

(7) Rinse off the stain with 2 per cent. solution of sodium chlorid, mount in this solution, and examine with a one-twelfth objective.

Buerger suggests a very useful variation as follows: After the alcohol wash and drying, the specimen is stained by Gram's method (p. 409), counterstained with aqueous solution of fuchsin, washed, and mounted in water. The pneumococcus holds the purple stain, while all capsules take on the pink counterstain.

Smith's Method.—(1) Make thin smears of the sputum or other material, which should be as fresh as possible.

(2) Fix in the flame in the usual manner.

(3) Apply a 10 per cent. aqueous solution of phospho-molybdic acid (Merck) for four to five seconds.

(4) Rinse in water.

(5) Apply anilin-gentian-violet, steaming gently for fifteen to thirty seconds.

(6) Rinse in water.

(7) Apply Gram's iodin solution, steaming gently for fifteen to thirty seconds.

(8) Wash in 95 per cent. alcohol until the purple color ceases to come off.

(9) Rinse in water.

(10) Apply a 6 per cent. aqueous solution of eosin (Grübler, w. g.), and gently warm for one-half to one minute.

(11) Rinse in water.

(12) Wash in absolute alcohol.

(13) Clear in xylol.

(14) Mount in balsam.

This is essentially Gram's method (see p. 409), preceded by treatment with phosphomolybdic acid and followed by eosin. Gram-positive bacteria like the pneumococcus are deep purple; capsules are pink and stand out clearly.

When the method is applied to Gram-negative bacteria, steps 5 to 9 inclusive are omitted; between steps 11 and 12 the preparation is counterstained with Löffler's methylene-blue, gently warming for fifteen to thirty seconds.

Anilin-gentian-violet.—Ehrlich's formula is the one generally used, but this keeps only a few weeks. Stirling's solution, which keeps much better and seems to give equal results, is as follows: gentian-violet, 5 gm.; alcohol, 10 c.c.; anilin oil, 2 c.c.; water, 88 c.c.

Formalin-gentian-violet is a satisfactory substitute for anilin-gentian-violet and is permanent. It consists of 5 per cent. solution formalin, 75 parts; saturated alcoholic solution gentian-violet, 25 parts.

Gram's Iodin Solution.—Iodin, 1 gm.; potassium iodid, 2 gm.; water, 300 c.c.

Löffler's alkaline methylene-blue is a very generally useful stain for bacteria. It is composed of 30 parts of a saturated alcoholic solution of methylene-blue and 100 parts of a 1 : 10,000 aqueous solution of caustic potash. It keeps indefinitely.

(4) **Bacillus of Friedländer (Bacillus Mucosus Capsulatus).**—In a small percentage of cases of pneumonia this organism is found alone or in company with the pneumococcus. Its pathologic significance is uncertain. It is often present in the respiratory tract under normal conditions. Friedländer's bacilli are non-motile, encapsulated rods, sometimes arranged in short chains (Fig. 13). Very short individuals in pairs closely resemble pneumococci, from which they are distinguished by the fact that they are Gram-decolorizing.

Fig. 13.—Friedländer's bacillus in pus from pulmonary abscess (obj. one-twelfth) (Boston).

(5) **Bacillus of Influenza.**—This is the etiologic factor in true influenza, although conditions which are clinically similar or identical may be caused by the pneumococcus, streptococcus, or *Micrococcus catarrhalis*. It is present, often in large numbers, in the nasal and bronchial secretions, and is also found in the local lesions following influenza. Chronic infection by influenza bacilli may be mistaken clinically for tuberculosis, and they should be searched for in all cases of obstinate chronic bronchitis.

Their recognition depends upon the facts that they are extremely small bacilli; that most of them lie within the pus-cells; that their ends stain more deeply than their centers, sometimes giving the appearance of minute diplococci; and that they are decolorized by Gram's method of staining (Figs. 14 and 149).

They are stained blue in the methods for tubercle bacilli, but are more certainly recognized by Gram's

method, followed by a counterstain. Pappenheim's pyronin-methyl-green stain is especially satisfactory.

(6) **Micrococcus Catarrhalis.**—This organism is frequently present in the sputum in inflammatory conditions of the respiratory tract resembling influenza. It is sometimes present in the nasal secretions in health. It is a Gram-negative diplococcus, frequently intracellular, and can be distinguished from the meningo-

Fig. 14.—Bacillus of influenza; cover-glass preparation of sputum from a case of influenza, showing the bacilli in leukocytes; highly magnified (Pfeiffer).

coccus and gonococcus only by means of cultures. It grows readily on ordinary media.

2. Cells.—These include pus-corpuscles, epithelial cells, and red blood-corpuscles.

(1) **Pus-corpuscles** are present in every sputum, and at times the sputum may consist of little else. They are the polymorphonuclear leukocytes of the blood, and appear as rounded cells with several nuclei or one very irregular nucleus (Fig. 11 and Plate II, Fig. 2). They

are frequently filled with granules of coal-dust and are often much degenerated. Such coal-dust-laden leuko-cytes are especially abundant in anthracosis, where angular black particles, both intra- and extra-cellular, are often so numerous as to color the sputum (Plate II, Fig. 2, *B*). Occasionally mononuclear leukocytes are present.

Eosinophilic cells are quite constantly found in large numbers in the sputum of bronchial asthma near the

Fig. 15.—Sputum from a case of asthma showing leukocytes, some containing eosinophilic granules; free eosinophilic granules and micrococci; stained with eosin and methylene-blue (× 350) (Jakob).

time of the paroxysm, and constitute one of the most distinctive features of the sputum of this disease. They resemble ordinary pus-corpuscles, except that their cytoplasm is filled with coarse granules having a marked affinity for eosin. It is worthy of note that many of them, sometimes the majority, are mononuclear. Large numbers of free granules, derived from disintegrated cells, are also found (Fig. 15).

Ordinary pus-cells are easily recognized in sputum stained by any of the methods already given. For eosinophilic cells, some method which includes eosin must be used. A simple method is to stain the dried and fixed film two or three minutes with saturated solution of eosin, and then one-half to one minute with Löffler's methylene-blue; nuclei and bacteria will be blue, eosinophilic granules bright red.

(2) **Epithelial cells** may come from any part of the respiratory tract. A few are always present, since desquamation of cells goes on constantly. Their recognition is important chiefly as an aid in deciding upon the source of the portion of the sputum in which they are found. In suspected lung conditions it is manifestly useless to study material from the nose only, yet this is not infrequently done. They have little diagnostic value, although a considerable excess would indicate a pathologic condition at the site of their origin. Any of the stains mentioned above will show them, and they can usually be identified in unstained sputum. In general, three forms are found:

(a) *Squamous Cells.*—Large, flat, polygonal cells with a comparatively small nucleus (Fig. 16, *i*). They come from the upper air-passages, and are especially numerous in laryngitis and pharyngitis. They are frequently studded with bacteria—most commonly diplococci.

(b) *Cylindric Cells from the Nose, Trachea, and Bronchi* (Fig. 16, *f*, *h*).—These are not usually abundant, and, as a rule, they are not identified because much altered from their original form, being usually round and swollen. When very fresh, they may retain their cylindric form, sometimes bearing cilia in active motion.

(*c*) *Alveolar Cells.*—Rather large, round, or oval cells with one or two round nuclei (Fig. 16). Their source is presumably the pulmonary alveoli. Like the leukocytes, they frequently contain particles of carbon (normal lung pigment). In chronic heart disease, owing to long-continued passive congestion, they may be filled
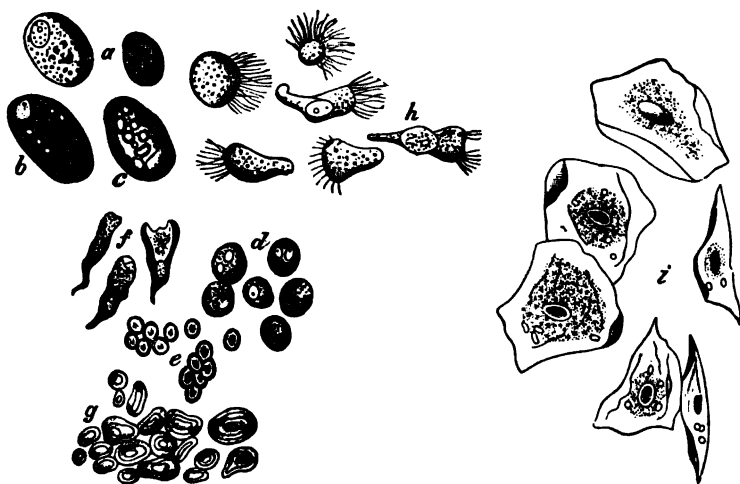

Fig. 16.—Different morphologic elements of the sputum (unstained): *a, b, c,* Pulmonary or alveolar epithelium—*a,* with normal lung pigment (carbon); *b,* with fat-droplets; *c,* with myelin globules; *d,* pus-corpuscles; *e,* red blood-corpuscles; *f,* cylindric beaker-shaped bronchial epithelial cells; *g,* free myelin globules; *h,* ciliated epithelium of different kinds from the nose, altered by coryza; *i,* squamous cells from the pharynx (after Bizzozero).

with brown granules of altered blood-pigment, and are then called "heart-failure cells." The presence of these cells in considerable numbers, by directing one's attention to the heart, will sometimes clear up the etiology of a chronic bronchitis. They are best seen in unstained sputum, appearing as grayish or colorless

balls filled with rounded granules of brown or yellow pigment. (Plate II, Fig. 1.)

Alveolar cells commonly contain fat-droplets and, less frequently, myelin globules. The latter are colorless, rounded bodies, sometimes resembling fat-droplets, but often showing concentric or irregularly spiral markings (Fig. 16, *c*, *g*). They are also found free in the sputum. They are abundant in the scanty morning sputum of apparently healthy persons, but may be present in any mucoid sputum.

(3) **Red blood-corpuscles** may be present in small numbers in almost any sputum. When fairly constantly present in considerable numbers, they are suggestive of phthisis. The corpuscles, when fresh, are shown by any of the staining methods which include eosin. They are commonly so much degenerated as to be unrecognizable, and often only altered blood-pigment is left. Ordinarily, blood in the sputum is sufficiently recognized with the naked eye.

III. CHEMIC EXAMINATION

There is little to be learned from a chemic examination, and it is rarely undertaken. Recently, however, it has been shown that the presence or absence of albumin may have clinical significance. Albumin is constantly present in the sputum in pneumonia, pulmonary edema, and tuberculosis. It is usually absent in bronchitis. A test for albumin may, therefore, be of great value in distinguishing between bronchitis and tuberculosis, a negative result practically proving the absence of tuberculosis. It is carried out as follows: The sputum is acidified with acetic acid to precipitate mucin and fil-

tered. If tenacious, it is first shaken up with water. The filtrate is then tested for albumin, as described in the chapter upon the Urine.

IV. THE SPUTUM IN DISEASE

Strictly speaking, any appreciable amount of sputum is abnormal. A great many healthy persons, however, raise a small quantity each morning, owing chiefly to the irritation of inhaled dust and smoke. Although not normal, this can hardly be spoken of as pathologic. It is particularly frequent in city dwellers and in those who smoke cigarettes to excess. In the latter, the amount is sometimes so great as to arouse suspicion of tuberculosis. Such " normal morning sputum " generally consists of small, rather dense, mucoid masses, translucent white, or, when due to inhaled smoke, gray in color. Microscopically, there are a few pus-corpuscles, and, usually, many alveolar cells, both of which may contain carbon particles. The alveolar cells commonly show myelin degeneration, and free myelin globules may be present in large numbers. Saprophytic bacteria may be present, but are not abundant.

1. Acute Bronchitis.—There is at first a small amount of tenacious, almost purely mucoid sputum, frequently blood streaked. This gradually becomes more abundant, mucopurulent in character, and yellowish or gray in color. At first the microscope shows a few leukocytes and alveolar and bronchial cells; later, the leukocytes become more numerous. Bacteria are not usually abundant.

2. Chronic Bronchitis.—The sputum is usually abundant, mucopurulent, and yellowish or yellowish-green in

color. Nummular masses—circular, " coin-like " discs which sink in water—may be seen. Microscopically, there are great numbers of leukocytes, often much degenerated. Epithelium is not abundant. Bacteria of various kinds, especially staphylococci, are usually numerous.

In fibrinous bronchitis there are found, in addition, fibrinous casts, usually of medium size.

In the chronic bronchitis accompanying long-continued passive congestion of the lungs, as in poorly compensated heart disease, the sputum may assume a rusty brown color, owing to presence of large numbers of the " heart-failure cells " previously mentioned.

3. Bronchiectasis.—When there is a single large cavity, the sputum is very abundant at intervals,—sometimes as high as a liter in twenty-four hours,—and has a very offensive odor. It is thinner than that of chronic bronchitis, and upon standing separates into three layers of pus, mucus, and frothy serum. It contains great numbers of miscellaneous bacteria.

4. Gangrene of the Lung.—The sputum is abundant, fluid, very offensive, and brownish in color. It separates into three layers upon standing—a brown deposit, a clear fluid, and a frothy layer. Microscopically, few cells of any kind are found. Bacteria are extremely numerous; among them may sometimes be found an acid-fast bacillus probably identical with the smegma bacillus. As before stated, elastic fibers are usually present in large fragments.

5. Pulmonary Edema.—Here there is an abundant, watery, frothy sputum, varying from faintly yellow or pink to dark brown in color; a few leukocytes and

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epithelial cells and varying numbers of red blood-corpuscles are found with the microscope.

6. Bronchial Asthma.—The sputum during and following an attack is scanty and very tenacious. Most characteristic is the presence of Curschmann's spirals, Charcot-Leyden crystals, and eosinophilic leukocytes.

7. Croupous Pneumonia.—Characteristic of this disease is a scanty, rusty red, very tenacious sputum, containing red corpuscles or altered blood-pigment, leukocytes, epithelial cells, usually many pneumococci, and often very small fibrinous casts. This sputum is seen during the stage of red hepatization. During resolution the sputum assumes the appearance of that of chronic bronchitis. When pneumonia occurs during the course of a chronic bronchitis, the characteristic rusty red sputum may not appear.

8. Pulmonary Tuberculosis.—The sputum is variable. In the earliest stages it may be scanty and almost purely mucoid, with an occasional yellow flake, or there may be only a very small mucopurulent mass. When the quantity is very small, there may be no cough, the sputum reaching the larynx by action of the bronchial cilia. This is not well enough recognized by practitioners. A careful inspection of all the sputum brought up by the patient on several successive days, and a microscopic examination of all yellow portions, will not infrequently establish a diagnosis of tuberculosis when physical signs are negative. Tubercle bacilli will sometimes be found in large numbers at this stage. Blood-streaked sputum is strongly suggestive of tuberculosis, and is more common in the early stages than later.

The sputum of more advanced cases resembles that of

chronic bronchitis, with the addition of tubercle bacilli and elastic fibers. Caseous particles containing immense numbers of the bacilli are common. Far-advanced cases with large cavities often show rather firm, spheric or ovoid masses of thick pus in a thin fluid —the so-called " globular sputum." These globular masses usually contain many tubercle bacilli. Considerable hemorrhages are not infrequent, and for some time thereafter the sputum may contain clots of blood or be colored brown.

CHAPTER II

THE URINE

Preliminary Considerations.—The urine is an aqueous solution of various organic and inorganic substances. It is probably both a secretion and an excretion. Most of the substances in solution are either waste-products from the body metabolism or products derived directly from the foods eaten. Normally, the total amount of solid constituents carried off in twenty-four hours is about 60 gm., of which the organic substances make up about 35 gm. and the inorganic about 25 gm.

The most important organic constituents are urea, uric acid, and ammonia. Urea constitutes about one-half of all the solids, or about 30 gm. in twenty-four hours.

The chief inorganic constituents are the chlorids, phosphates, and sulphates. The chlorids, practically all in the form of sodium chlorid, make up about one-half of the inorganic substances, or about 13 gm., in twenty-four hours.

Certain substances appear in the urine only in pathologic conditions. The most important of these are proteins, sugars, acetone, and related substances, bile, hemoglobin, and the diazo substances.

In addition to the substances in solution all urines contain various microscopic structures.

While, under ordinary conditions, the composition of

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urine does not vary much from day to day, it varies greatly at different hours of the same day. It is evident, therefore, that *no quantitative test can be of value unless a sample of the mixed twenty-four-hour urine be used.* The patient should be instructed to void all the urine during the twenty-four hours into a clean vessel kept in a cool place, to mix it well, to measure the whole quantity, and to bring eight or more ounces for examination. A pint fruit-jar is a convenient container. When it is desired to make only qualitative tests, as for albumin or sugar, a " sample " voided at random will answer. It should be remembered, however, that urine passed about three hours after a meal is most likely to contain pathologic substances. That voided first in the morning is least likely to contain them. To diagnose cyclic albuminuria samples obtained at various periods during the twenty-four hours must be examined.

The urine must be examined while fresh. Decomposition sets in rapidly, especially in warm weather, and greatly interferes with all the examinations. Decomposition may be delayed by adding five grains of boric acid (as much of the powder as can be heaped upon a ten-cent piece) for each four ounces of urine. Formalin, in proportion of one drop to four ounces, is also an efficient preservative, but if larger amounts be used, it may give reactions for sugar and albumin, and is likely to cause a precipitate which greatly interferes with the microscopic examination.

Normal and abnormal pigments, which interfere with certain of the tests, can be removed by filtering the urine through animal charcoal, or precipitating with a solution of acetate of lead and filtering.

Certain cloudy urines cannot be clarified by ordinary filtration through paper, particularly when the cloudiness is due to bacteria. Such urines can usually be rendered perfectly clear by adding a small amount of purified talc, shaking well, and filtering.

A suspected fluid can be identified as urine by detecting any considerable quantity of urea in it (p. 93). Traces of urea may, however, be met with in ovarian cyst fluid, while urine from very old cases of hydronephrosis may contain little or none.

The frequency of micturition is often suggestive in diagnosis. Whether it is unduly frequent can best be ascertained by asking the patient whether he has to get up at night to urinate. Increased frequency may be due to restlessness; to increased quantity of urine; to irritability of the bladder, usually an evidence of cystitis; to obstruction (" retention with overflow "); or to paralysis of the sphincter.

Clinical examination of the urine may conveniently be considered under four heads: I. Physical examination. II. Chemic examination. III. Microscopic examination. IV. The urine in disease.

I. PHYSICAL EXAMINATION

1. Quantity.—The quantity passed in twenty-four hours varies greatly with the amount of liquids ingested, perspiration, etc. The normal may be taken as 1000 to 1500 c.c., or 40 to 50 ounces.

The quantity is increased (polyuria) during absorption of large serous effusions and in many nervous conditions. It is usually much increased in chronic interstitial nephritis, diabetes insipidus, and diabetes mellitus. In these

conditions a permanent increase in amount of urine is characteristic—a fact of much value in diagnosis. In diabetes mellitus the urine may, though rarely, reach the enormous amount of 50 liters.

The quantity is decreased (oliguria) in severe diarrhea; in fevers; in all conditions which interfere with circulation in the kidney, as poorly compensated heart disease; and in the parenchymatous forms of nephritis. In uremia the urine is usually very greatly decreased and may be entirely suppressed (anuria).

2. Color.—This varies considerably in health, and depends largely upon the quantity of urine voided. The usual color is yellow or reddish-yellow, due to the presence of several pigments, chiefly urochrome. Acid urine is generally darker than alkaline. In recording the color Vogel's scale (see *Frontispiece*) is very widely used, the urine being filtered and examined by transmitted light in a glass three or four inches in diameter.

The color is sometimes greatly changed by abnormal pigments. Blood-pigment gives a red or brown, smoky color. Urine containing bile is yellowish or brown, with a yellow foam when shaken. It may assume a greenish hue after standing, owing to oxidation of bilirubin into biliverdin. Ingestion of small amounts of methylene-blue gives a pale green; large amounts give a marked blue. Santonin produces a yellow; rhubarb, senna, cascara, and some other cathartics, a brown color; these change to red upon addition of an alkali, and if the urine be alkaline when voided, may cause suspicion of hematuria. Thymol gives a yellowish-green. Following poisoning from phenol and related drugs the urine may have a normal color when voided, but becomes olive-

green to brownish-black upon standing. In susceptible individuals therapeutic doses of creosote, or absorption from carbolized dressings, may cause this change. Urine which contains melanin, as sometimes in melanotic sarcoma, and very rarely in wasting diseases, also becomes brown or black upon long standing. A similar darkening upon exposure to the air occurs in alkaptonuria (p. 126).

A pale greenish urine with high specific gravity strongly suggests diabetes mellitus.

3. Transparency.—Freshly passed normal urine is clear. Upon standing, a faint cloud of mucus, leukocytes, and epithelial cells settles to the bottom—the so-called "nubecula." Abnormal cloudiness is usually due to presence of phosphates, urates, pus, blood, or bacteria.

Amorphous phosphates are precipitated in neutral or alkaline urine. They form a white cloud and sediment, which disappear upon addition of an acid.

Amorphous urates are precipitated only in acid urine. They form a white or pink cloud and sediment (" brickdust deposit "), which disappear upon heating.

Pus resembles amorphous phosphates to the naked eye. Its nature is easily recognized with the microscope, or by adding a strong solution of caustic soda to the sediment, which is thereby transformed into a gelatinous mass (Donné's test).

Blood gives a reddish or brown, smoky color, and may be recognized with the microscope or by tests for hemoglobin.

Bacteria, when present in great numbers, give a uniform cloud, which cannot be removed by ordinary filtration. They are detected with the microscope.

The cloudiness of decomposing urine is due mainly to precipitation of phosphates and multiplication of bacteria.

4. Odor.—The characteristic aromatic odor is due to volatile acids, and is most marked in concentrated urines. During decomposition, the odor becomes ammoniacal. A fruity odor is sometimes noted in diabetes, due probably to acetone. Urine which contains cystin may develop an odor of sulphureted hydrogen during decomposition.

Various articles of diet and drugs impart peculiar odors. Notable among these are asparagus, which gives a characteristic offensive odor, and turpentine, which imparts an odor somewhat suggesting that of violets.

5. Reaction.—Normally, the mixed twenty-four-hour urine is slightly acid in reaction. The acidity was formerly held to be due to acid phosphates, but Folin has shown that the acidity of a clear urine is ordinarily much greater than the acidity of all the phosphates, the excess being due to free organic acids. Individual samples may be slightly alkaline, especially after a full meal, or amphoteric. The reaction is determined by means of litmus-paper.

Acidity is increased after administration of certain drugs, and whenever the urine is concentrated from any cause, as in fevers. A very acid urine may cause frequent micturition because of its irritation. This is often an important factor in the troublesome enuresis of children.

The urine always becomes alkaline upon long standing, owing to decomposition of urea with formation of ammonia. If markedly alkaline when voided, it usually

indicates such "ammoniacal decomposition" in the bladder, which is the rule in chronic cystitis, especially that due to paralysis or obstruction. Alkalinity due to ammonia (*volatile alkalinity*) can be distinguished by the fact that litmus paper turned blue by the urine again becomes red upon gentle heating. *Fixed alkalinity* is due to alkaline salts, and is often observed during frequent vomiting, after the crisis of pneumonia, in various forms of anemia, after full meals, and after administration of certain drugs, especially salts of vegetable acids.

Quantitative estimation of the acidity of urine is not of much clinical value. When, however, it is desired to make it, the method of Folin will be found satisfactory. In every case the sample must be from the mixed twenty-four-hour urine and as fresh as possible.

Folin's Method.—Into a small flask measure 25 c.c. of the urine and add 1 or 2 drops 0.5 per cent. alcoholic solution of phenolphthalein and 15 or 20 gm. of neutral potassium oxalate. Shake for a minute, and immediately titrate with decinormal sodium hydroxid, shaking meanwhile, until the first permanent pink appears. Read off from the buret the amount of decinormal sodium hydroxid solution added, and calculate the number of cubic centimeters which would be required for the entire twenty-four hours' urine. Folin places the normal acidity, obtained in this way, at 617.

6. Specific Gravity.—The normal average is about 1.017 to 1.020. Samples of urine taken at random may go far above or below these figures, hence a sample of the mixed twenty-four-hour urine should always be used.

Pathologically, it may vary from 1.001 to 1.060. It is *low* in chronic interstitial nephritis, diabetes insipidus,

and many functional nervous disorders. It is *high* in fevers and in parenchymatous forms of nephritis. In any form of nephritis a sudden fall without a corresponding increase in quantity of urine may foretell approaching uremia. It is *highest* in diabetes mellitus. A high specific gravity when the urine is not highly colored should lead one to suspect this disease. A normal specific gravity does not, however, exclude it.
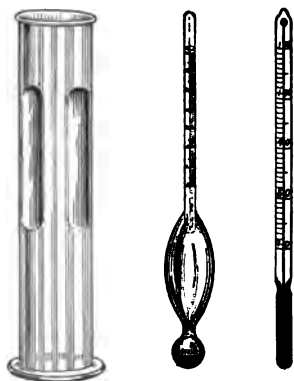
Fig. 17.—Squibb's urinometer with thermometer and cylinder.

The specific gravity is most conveniently estimated by means of the urinometer—Squibb's is preferable (Fig. 17). It is standardized for a temperature of 77° F., and the urine should be at or near that temperature. Care should be taken that the urinometer does not touch the side of the tube, and that air-bubbles are removed from the surface of the urine. With most instruments the reading is taken from the bottom of the meniscus. A long scale on the stem is desirable, because of the greater ease of accurate reading.

One frequently wishes to ascertain the specific gravity of quantities of fluid too small to float an urinometer. A simple device for this purpose, which requires only about 3 c.c. and is very satisfactory in clinical work has been designed by Saxe (Fig. 18).   The urine is placed in the bulb at the bottom, the instrument is floated in dis-
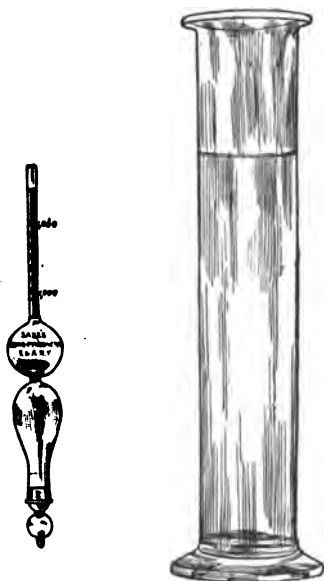
Fig. 18.—Saxe's urinopyknometer and jar for same.

tilled water, and the specific gravity is read off from the scale upon the stem.

7. Total Solids.—An estimation of the total amount of solids which pass through the kidneys in twenty-four hours is, in practice, one of the most useful of urinary examinations.   The normal for a man of 150 pounds is about 60 gm., or 950 gr.   The principal factors which

influence this amount are body weight (except with excessive fat), diet, exercise, and age, and these should be considered in making an estimation. After about the forty-fifth year it becomes gradually less; after seventy-five years it is about one-half the amount given.

In disease, the amount of solids depends mainly upon the activity of metabolism and the ability of the kidneys to excrete. An estimation of the solids, therefore, furnishes an important clue to the functional efficiency of the kidneys. The kidneys bear much the same relation to the organism as does the heart: they cause no direct harm so long as they are capable of performing the work required of them. When, however, through either organic disease or functional inactivity, they fail to carry off their proportion of the waste-products of the body, some of these products must either be eliminated through other organs, where they cause irritation and disease, or be retained within the body, where they act as poisons. The great importance of these poisons in production of distressing symptoms and even organic disease is not well enough recognized by most practitioners. Disappearance of unpleasant and perplexing symptoms as the urinary solids rise to the normal under proper treatment is often most surprising.

When, other factors remaining unchanged, the amount of solids eliminated is considerably above the normal, increased destructive metabolism may be inferred.

The total solids can be estimated roughly, but accurately enough for most clinical purposes, by multiplying the last two figures of the specific gravity of the mixed twenty-four-hour urine by the number of ounces

voided and to the product adding one-tenth of itself. This gives the amount in grains. If, for example, the twenty-four-hour quantity is 3 pints or 48 ounces, and the specific gravity is 1.018, the total solids would approximate 950 gr., as follows:

$$48 \times 18 = 864; 864 + 86.4 = 950.4$$

This method is especially convenient for the practitioner, because patients nearly always report the amount of urine in pints and ounces, and it avoids the necessity of converting into the metric system. Häser's method is more widely used but is less convenient. The last two figures of the specific gravity are multiplied by 2.33. The product is then multiplied by the number of cubic centimeters voided in twenty-four hours and divided by 1000. This gives the total solids in grams.

8. Functional Tests.—Within the past few years much thought has been devoted to methods of more accurately ascertaining the functional efficiency of the kidneys, especially of one kidney when removal of the other is under consideration. The most promising of the methods which have been devised are cryoscopy, electric conductivity, the methylene-blue test, and the phloridzin test. It is doubtful whether, except in experienced hands, these yield any more information than can be had from an intelligent consideration of the specific gravity and the twenty-four-hour quantity, together with a microscopic examination. They are most useful when the urines obtained from separate kidneys by segregation or ureteral catheterization are compared. The reader is referred to larger works upon urinalysis for details.

Cryoscopy, determination of the freezing-point, depends upon the principle that the freezing-point of a fluid is depressed in proportion to the number of molecules, organic and inorganic, in solution. To have any value, the freezing-point of the urine must be compared with that of the blood, since it is not so much the number of molecules contained in the urine as the number which the kidney has failed to carry off and has left in the blood, that indicates its insufficiency.

Electric conductivity refers to the power of the urine to carry an electric current. It is increased in proportion to the number of *inorganic* molecules in solution.

In the **methylene-blue test** of Achard and Castaigne a solution of methylene-blue is injected intramuscularly, and the time of its appearance in the urine is noted. Normally, it appears in about thirty minutes. When delayed, renal " permeability " is supposed to be interfered with. Since methylene-blue is sometimes excreted as a colorless derivative, indigo-carmin has been proposed as a substitute. In the absence of renal insufficiency this always gives a blue color, which begins to appear in about five minutes.

The **phloridzin test** consists in the hypodermic injection of a small quantity of phloridzin. This substance is transformed into glucose by the kidneys of healthy persons. In disease, this change is more or less interfered with, and the amount of glucose recoverable from the urine is taken as an index of the secretory power of the kidneys.

In applying these tests for " permeability," " secretory ability," etc., one must remember that the conditions are abnormal, and that there is no evidence that

the kidneys will behave with the products of metabolism as they do with the substances selected for the tests, and also that the tests throw unusual work upon the kidneys, which in some cases may be harmful.

II. CHEMIC EXAMINATION

A. NORMAL CONSTITUENTS

Of the large number of organic and inorganic substances normally present in the urine, only a few demand any consideration from the clinician. The following table, therefore, outlines the average composition from the clinical, rather than from the chemical, standpoint. Only the twenty-four-hour quantities are given, since they alone furnish an accurate basis for comparison. *The student cannot too soon learn that percentages mean little or nothing, excepting as they furnish a means of calculating the twenty-four-hour elimination.*

COMPOSITION OF NORMAL URINE

	Grams in twenty-four hours.	Approximate average.
Total substances in solution	55–70	60
Inorganic substances	20–30	25
Chlorids (chiefly sodium chlorid)	10–15	12.5
Phosphates (estimated as phosphoric acid), total	2.5–3.5	3
Earthy $\frac{1}{3}$ of total		1
Alkaline $\frac{2}{3}$ of total		2
Sulphates (estimated as sulphuric acid), total	1.5–3.0	2.5
Mineral $\frac{9}{10}$ of total		2.25
Conjugate $\frac{1}{10}$ of total		0.25
Includes indican		Trace
Ammonia	0.5–1.0	0.7
Organic substances	30–40	35
Urea	20–35	30
Uric acid	0.4–1.0	0.7
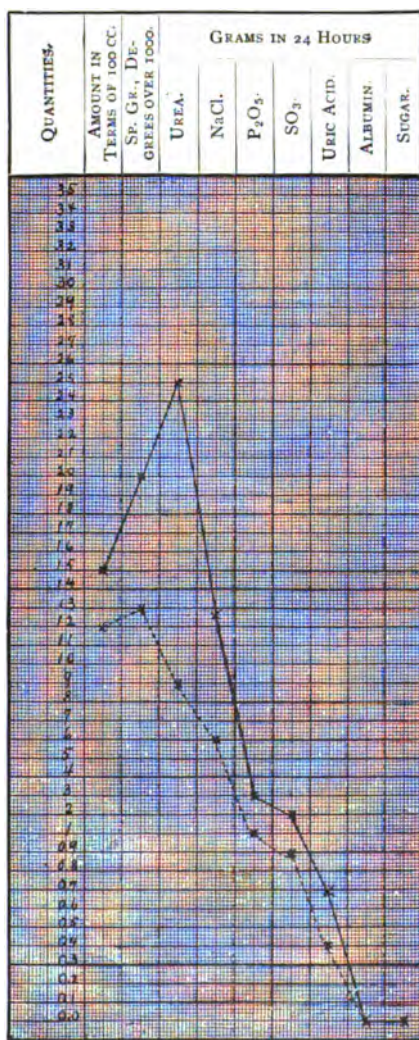
Fig. 19.—Graphic expression of quantities in the urine. Solid line, normal urine; dotted line, an example of pathologic urine in a case of cancerous cachexia (Saxe).

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Although the conjugate sulphates are organic compounds, they are, for the sake of convenience, included with the inorganic sulphates in the table on p. 80.

Among constituents which are of little clinical importance, or are present only in traces, are:

Inorganic: Iron, carbonates, nitrates, silicates, and fluorids.

Organic: Creatinin, hippuric acid, purin bases, oxalic acid, benzoic acid, volatile fatty acids, pigments, and acetone.

Variations in body weight, diet, and exercise cause marked fluctuations in the total solids and in individual substances.

1. Chlorids.—These are derived from the food, and are mainly in the form of sodium chlorid. The amount excreted normally is 10 to 15 gm. in twenty-four hours. It is much affected by the diet, and is reduced to a minimum in starvation.

Excretion of chlorids is diminished in nephritis and in fevers, especially in pneumonia and inflammations leading to the formation of large exudates. In nephritis the kidneys are less permeable to the chlorids, and it is possible that the edema is due largely to an effort of the body to dilute the chlorids which have been retained. Certainly an excess of chlorids in the food will increase both the albuminuria and the edema of nephritis. In fevers the diminution is due largely to decrease of food, though probably in some measure to impaired renal function. In pneumonia chlorids are constantly very low, and in some cases are absent entirely. Following the crisis they are increased. In inflammations leading to formation of large exudates—*e. g.*, pleurisy with

effusion—chlorids are diminished, because a considerable amount becomes "locked up" in the exudate. During absorption chlorids are liberated and appear in the urine in excessive amounts.

Diminution of chlorids is also observed in severe diarrhea, anemic conditions, and carcinoma of the stomach.

Fig. 20.—The Purdy electric centrifuge with four arms.

Quantitative Estimation.—The best method for clinical purposes is the centrifugal method.

Purdy's Centrifugal Methods.—As shown by the late Dr. Purdy, the centrifuge offers an important means of making quantitative estimations of a number of substances in the urine. Results are easily and quickly obtained, and are probably accurate enough for all clinical purposes.

In general, the methods consist in precipitating the substance to be estimated in a graduated centrifuge tube,

and applying a definite amount of centrifugal force for a definite length of time, after which the percentage of precipitate is read off upon the side of the tube. Albumin, if present, must be previously removed by boiling and filtering. Results are in terms of *bulk of precipitate*, which must not be confused with *percentage by weight*. The weight percentage can be found by referring to Purdy's tables, given later. In this, as in

Fig. 21.—Water-motor centrifuge.

all quantitative urine work, percentages mean little in themselves; the actual amount eliminated in twenty-four hours should always be calculated.

The centrifuge should have an arm with radius of 6¾ inches when in motion, and should be capable of maintaining a speed of 1500 revolutions a minute. The electric centrifuge is to be recommended, although good work can be done with a water-power centrifuge, or, after a little practice, with the hand centrifuge. A

speed indicator is desirable with electric and water-motor machines, although one can learn to estimate the speed by the musical note.
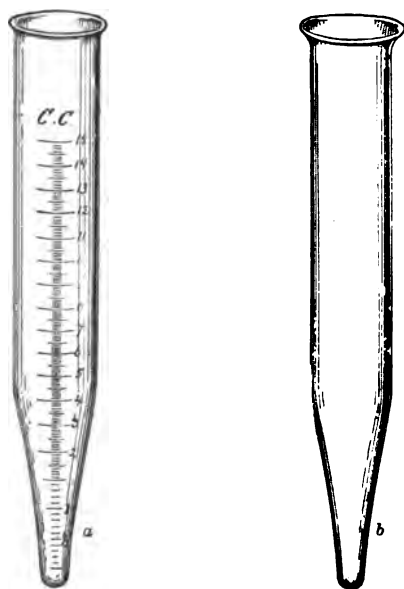
Fig. 22.—Purdy's tubes for the centrifuge: *a*, Percentage tube; *b*, sediment tube.

Estimation of Chlorids.—Fill the graduated tube to the 10 c.c. mark with urine; add 15 drops strong nitric acid and then silver nitrate solution (dram to the ounce) to the 15 c.c. mark. Mix by inverting several times. Let stand a few minutes for a precipitate to form, and then revolve in the centrifuge for three minutes at 1200 revolutions a minute. Each one-tenth cubic centimeter of precipitate equals 1 per cent. by bulk. The normal is about 10 per cent. This may be converted into terms of chlorin or sodium chlorid by means of the table upon page 86. Roughly speaking, the percent-

age of chlorin by weight is about one-twelfth the bulk-percentage.

TABLE FOR THE ESTIMATION OF CHLORIDS AFTER CENTRIFUGATION

Showing the bulk-percentage of silver chlorid (AgCl) and the corresponding gravimetric percentages and grains per fluidounce of sodium chlorid (NaCl) and chlorin (Cl).—(Purdy.)

Bulk-percentage of AgCl	Percentage NaCl	Gr. Per Oz. NaCl	Percentage Cl	Gr. Per Oz. Cl	Bulk-percentage of AgCl	Percentage NaCl	Gr. Per Oz. NaCl	Percentage Cl	Gr. Per Oz. Cl
¼	0.03	0.15	0.02	0.1	8	1.04	4.98	0.63	3.02
½	0.07	0.31	0.04	0.19	8½	1.1	5.29	0.67	3.22
¾	0.1	0.47	0.06	0.28	9	1.17	5.6	0.71	3.4
1	0.13	0.62	0.08	0.38	9½	1.23	5.91	0.75	3.6
1¼	0.16	0.78	0.1	0.48	10	1.3	6.22	0.79	3.79
1½	0.19	0.93	0.12	0.57	10½	1.36	6.53	0.83	3.97
1¾	0.23	1.09	0.14	0.67	11	1.43	6.84	0.87	4.16
2	0.26	1.24	0.16	0.76	11½	1.49	7.2	0.91	4.35
2¼	0.29	1.41	0.18	0.85	12	1.56	7.46	0.95	4.54
2½	0.32	1.56	0.2	0.96	12½	1.62	7.78	0.99	4.73
2¾	0.36	1.71	0.22	1.04	13	1.69	8.09	1.02	4.92
3	0.39	1.87	0.24	1.13	13½	1.75	8.4	1.06	5.11
3¼	0.42	2.02	0.26	1.23	14	1.82	8.71	1.1	5.29
3½	0.45	2.18	0.28	1.32	14½	1.88	9.02	1.14	5.49
3¾	0.49	2.35	0.3	1.42	15	1.94	9.33	1.18	5.67
4	0.52	2.49	0.32	1.51	15½	2.01	9.65	1.22	5.86
4¼	0.55	2.64	0.34	1.61	16	2.07	9.94	1.26	6.06
4½	0.58	2.8	0.35	1.7	16½	2.14	10.27	1.3	6.24
4¾	0.62	2.96	0.37	1.8	17	2.2	10.51	1.34	6.43
5	0.65	3.11	0.39	1.89	17½	2.27	10.87	1.38	6.62
5½	0.71	3.42	0.43	2.09	18	2.33	11.2	1.42	6.81
6	0.78	3.73	0.47	2.27	18½	2.4	11.51	1.46	7.0
6½	0.84	4.05	0.51	2.46	19	2.46	11.82	1.5	7.19
7	0.91	4.35	0.55	2.62	19½	2.53	12.13	1.54	7.38
7½	0.97	4.67	0.59	2.84	20	2.59	12.44	1.58	7.56

Bulk-percentage to be read on the side of the tube.

2. Phosphates.—Phosphates are derived largely from the food, only a small proportion resulting from metab-

olism. The normal daily output of phosphoric acid is
about 2.5 to 3.5 gm.

The urinary phosphates are of two kinds: *alkaline*,
which make up two-thirds of the whole, and include the
phosphates of sodium and potassium; and *earthy*, which
constitute one-third, and include the phosphates of cal-
cium and magnesium. Earthy phosphates are fre-
quently thrown out of solution in neutral and alkaline
urines, and as " amorphous phosphates " form a very
common sediment. This sediment seldom indicates an
excessive excretion of phosphoric acid. It is usually
merely an evidence of diminished acidity of the urine, or
of an increase in the proportion of phosphoric acid elimi-
nated as earthy phosphates. This form of " phospha-
turia " is most frequent in neurasthenia and hysteria.
When the urine undergoes ammoniacal decomposition,
some of the ammonia set free combines with magnesium
phosphate to form ammoniomagnesium phosphate
(" triple phosphate "), which is deposited in typical
crystalline form (p. 148).

Excretion of phosphates is *increased* by a rich diet;
in active metabolism; in certain nervous and mental dis-
orders; in leukemia; and in phosphatic diabetes, an ob-
scure disturbance of metabolism (not related to diabetes
mellitus) which is associated with an increase in the out-
put of phosphates up to 10 gm. or more in twenty-four
hours. Phosphates are *decreased* in chronic diseases
with lowered metabolism; in hepatic cirrhosis and acute
yellow atrophy; in pregnancy, owing to developing
fetal bones; and in nephritis, owing to kidney imper-
meability.

Quantitative estimation does not furnish much of

definite clinical value. The centrifugal method is the most convenient.

TABLE FOR THE ESTIMATION OF PHOSPHATES AFTER CENTRIFUGATION

Showing bulk-percentages of uranyl phosphate $(H[UO_2]PO_4)$ and the corresponding gravimetric percentages and grains per ounce of phosphoric acid (P_2O_5).—(Purdy.)

Bulk-percentage of $H(UO_2)PO_4$.	Percentage P_2O_5.	Gr. Per Oz. P_2O_5.	Bulk-percentage of $H(UO_2)PO_4$.	Percentage P_2O_5.	Gr. Per Oz. P_2O_5.
$\frac{1}{2}$	0.02	0.1	11	0.14	0.67
1	0.04	0.19	12	0.15	0.72
$1\frac{1}{2}$	0.045	0.22	13	0.16	0.77
2	0.05	0.24	14	0.17	0.82
$2\frac{1}{2}$	0.055	0.26	15	0.18	0.86
3	0.06	0.29	16	0.19	0.91
$3\frac{1}{2}$	0.065	0.31	17	0.2	0.96
4	0.07	0.34	18	0.21	1.
$4\frac{1}{2}$	0.075	0.36	19	0.22	1.06
5	0.08	0.38	20	0.23	1.1
6	0.09	0.43	21	0.24	1.15
7	0.1	0.48	22	0.25	1.2
8	0.11	0.53	23	0.26	1.25
9	0.12	0.58	24	0.27	1.3
10	0.13	0.62	25	0.28	1.35

Bulk-percentage to be read from graduation on the side of the tube.

Purdy's Centrifugal Method.—Take 10 c.c. urine in the graduated tube, add 2 c.c. of 50 per cent. acetic acid, and 3 c.c. of 5 per cent. uranium nitrate solution. Mix; let stand a few minutes, and revolve for three minutes at 1200 revolutions. The bulk of precipitate is normally about 8 per cent. The percentage of phosphoric acid by weight is, roughly, one-eighty-fifth of the bulk-percentage.

3. Sulphates.—The urinary sulphates are derived partly from the food, especially meats, and partly from body metabolism. The normal output of sulphuric acid is about 1.5 to 3 gm. daily. It is increased in condi-

tions associated with active metabolism, and in general may be taken as a rough index of protein metabolism.

Quantitative estimation of the total sulphates yields little of clinical value.

Purdy's Centrifugal Method.—Take 10 c.c. urine in the graduated tube and add barium chlorid solution to the 15 c.c. mark. This consists of barium chlorid, 4 parts; strong hydrochloric acid, 1 part; and distilled water, 16 parts. Mix; let stand a few minutes, and revolve for three minutes at 1200 revolutions a minute. The normal bulk of precipitate is about 0.8 per cent. The percentage by weight of sulphuric acid is about one-fourth of the bulk-percentage.

TABLE FOR THE ESTIMATION OF SULPHATES AFTER CENTRIFUGATION

Showing the bulk-percentages of barium sulphate ($BaSO_4$) and the corresponding gravimetric percentages and grains per fluidounce of sulphuric acid (SO_3).—(Purdy.)

Bulk-percentage of $BaSO_4$.	Percentage SO_3.	Gr. Per Oz. SO_3.	Bulk-percentage of $BaSO_4$.	Percentage SO_3.	Gr. Per Oz. SO_3.
⅛	0.04	0.19	2¼	0.55	2.64
¼	0.07	0.34	2½	0.61	2.93
⅜	0.1	0.48	2¾	0.67	3.22
½	0.13	0.62	3	0.73	3.5
⅝	0.16	0.77	3¼	0.79	3.79
¾	0.19	0.91	3½	0.85	4.08
⅞	0.22	1.06	3¾	0.91	4.37
1	0.25	1.1	4	0.97	4.66
1¼	0.31	1.49	4¼	1.03	4.94
1½	0.37	1.78	4½	1.09	5.23
1¾	0.43	2.06	4¾	1.15	5.52
2	0.49	2.35	5	1.21	5.81

Bulk-percentage to be read from graduation on the side of the tube.

About nine-tenths of the sulphuric acid is in combination with various mineral substances, chiefly sodium,

potassium, calcium, and magnesium (*mineral* or *pre-formed sulphates*). One-tenth is in combination with certain aromatic substances, which are mostly products of albuminous putrefaction in the intestine, but are derived in part from destructive metabolism (*conjugate* or *ethereal sulphates*). Among these aromatic substances are indol, phenol, and skatol. By far the most important of the conjugate sulphates and representative of the group is potassium indoxyl sulphate.

Potassium indoxyl sulphate, or **indican,** is derived from indol. Indol is absorbed and oxidized into indoxyl, which combines with potassium and sulphuric acid and is thus excreted. Under normal conditions the amount in the urine is small. It is increased by a meat diet.

Unlike the other ethereal sulphates, which are derived in part from metabolism, indican originates practically wholly from putrefactive processes. It alone, therefore, and not the total ethereal sulphates, can be taken as an index of such putrefaction. A pathologic increase is called indicanuria. It is noted in:

(*a*) *Diseases of the Small Intestine.*—This is by far the most common source. Intestinal obstruction gives the largest amounts of indican. It is also much increased in intestinal indigestion—so-called " biliousness "; in inflammations, especially in cholera and typhoid fever; and in paralysis of peristalsis, such as occurs in peritonitis. Simple constipation and diseases of the *large* intestine alone do not so frequently cause indicanuria.

(*b*) *Diseases of the stomach* associated with deficient hydrochloric acid, as chronic gastritis and gastric cancer.

Diminished hydrochloric acid favors intestinal putre-faction.

(c) *Diminished Flow of Bile.*—Since the bile serves both as a stimulant to peristalsis and an intestinal anti-septic, a diminished flow from any cause favors occur-rence of indicanuria.

(d) *Decomposition of exudates* anywhere in the body, as in empyema, bronchiectasis, and large tuberculous cavities.

Detection of indican depends upon its decomposition and oxidation of the indoxyl set free into indigo-blue. This change sometimes takes place spontaneously in decomposing urine, causing a dirty blue color. Crystals of indigo (Fig. 36) may be found both in the sediment and the scum.

Obermayer's Method.—In a test-tube take equal parts of the urine and Obermayer's reagent and add a small quantity of chloroform. Mix by inverting a few times; avoid shak-ing violently. If indican be present in excess, the chloroform, which sinks to the bottom, will assume an indigo-blue color. It will take up the indigo more quickly if the urine be warm. The depth of color indicates the comparative amount of indican if the same proportions of urine and reagents are always used, but one should bear in mind the total amount of urine voided. The indican in normal urine may give a faint blue by this method. Urine of patients taking iodids gives a reddish-violet color, which disappears upon addition of a few drops of strong sodium hyposulphite solution and shaking. Bile-pigments, which interfere with the test, must be removed (p. 69).

Obermayer's reagent consists of strong hydrochloric acid (sp. gr., 1.19), 1000 parts, and ferric chlorid, 2 parts. This makes a yellow, fuming liquid which keeps well.

4. Urea.—From the standpoint of physiology urea is the most important constituent of the urine. It is the principal waste-product of metabolism, and constitutes about one-half of all the solids excreted—about 20 to 35 gm. in twenty-four hours. It represents 85 to 90 per cent. of the total nitrogen of the urine, and its quantitative estimation is a simple, though not very accurate, method of ascertaining the state of nitrogenous excretion.

This is true, however, only in normal individuals upon average mixed diet. Under pathologic conditions, the proportion of nitrogen distributed among the various nitrogen-containing substances undergoes great variation. The only accurate index of protein metabolism is, therefore, the total output of nitrogen, which can be estimated by the Kjeldahl method. The whole subject of " nitrogen partition " and " nitrogen equilibrium " (relation of excretion to intake) is an important one, but is out of the province of this book, since as yet it concerns the physiologic chemist more than the clinician.

It may be helpful to state here, however, that upon a mixed diet the nitrogen of the urine is distributed about as follows: urea nitrogen, 86.9 per cent.; ammonia nitrogen, 4.4 per cent.; creatinin nitrogen, 3.6 per cent.; uric acid nitrogen, 0.75 per cent.; " undetermined nitrogen," chiefly in amino acids, 4.3 per cent.

Normally, the amount is greatly influenced by exercise and diet. It is increased by copious drinking of water and administration of ammonium salts of organic acids.

Pathologically, urea is increased in fevers, in diabetes, and especially during resolution of pneumonia and ab-

sorption of large exudates. As above indicated, when other factors are equal, the amount of urea indicates the activity of metabolism. In deciding whether in a given case an increase of urea is due to increased metabolism the relation between the amounts of urea and of the chlorids is a helpful consideration. The amount of urea is normally about twice that of the chlorids. If the proportion is much increased above this, increased tissue destruction may be inferred, since other conditions which increase urea also increase chlorids.

Urea is decreased in diseases of the liver with destruction of liver substance, such as cirrhosis, carcinoma, and acute yellow atrophy. It may or may not be decreased in nephritis. In the early stages of chronic nephritis, when diagnosis is difficult, it is usually normal. In the late stages, when diagnosis is comparatively easy, it is decreased. Hence estimation of urea is of little help in the diagnosis of this disease, especially when, as is so frequently the case, a small quantity of urine taken at random is used. When, however, the diagnosis is established, estimations made at frequent intervals under the same conditions of diet and exercise are of much value, *provided a sample of the mixed twenty-four-hour urine be used.* A steady decline is a very bad prognostic sign, and a sudden marked diminution is usually a forerunner of uremia.

The presence of urea can be shown by allowing a few drops of the fluid partially to evaporate upon a slide, and adding a small drop of pure, colorless nitric acid or saturated solution of oxalic acid. Crystals of urea nitrate or oxalate (Fig. 23) will soon appear and can be recognized with the microscope.

Quantitative Estimation.—The hypobromite method, which is generally used, depends upon the fact that urea is decomposed by sodium hypobromite with liberation of nitrogen. The amount of urea is calculated from
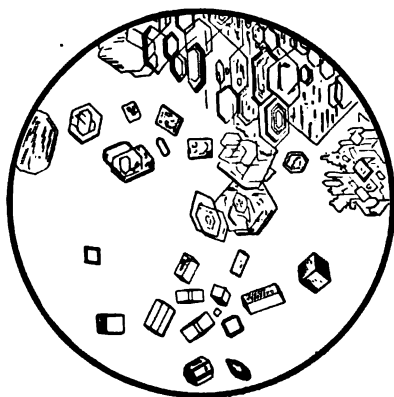
Fig. 23.—Crystals of nitrate of urea (upper half) and oxalate of urea (lower half) (after Funke).
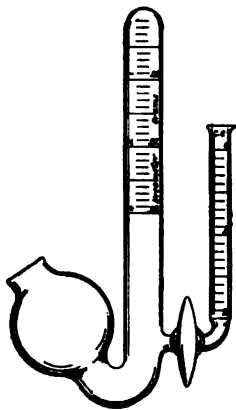
Fig. 24.—Doremus-Hinds' ureometer.

the volume of nitrogen set free. The improved Doremus apparatus (Fig. 24) is the most convenient.

Pour some of the urine into the smaller tube of the apparatus, then open the stopcock and quickly close it so as to fill its lumen with urine. Rinse out the larger tube with water and fill it and the bulb with 25 per cent. caustic soda solution. Add to this 1 c.c. of bromin by means of a medicine-dropper and mix well. This prepares a fresh solution of sodium hypobromite with excess of caustic soda, which serves to absorb the carbon dioxid set free in the decomposition of urea. When handling bromin, keep an open vessel of ammonia near to neutralize the irritant fumes.

Pour the urine into the smaller tube, and then turn the stopcock so as to let as much urine as desired (usually 1 c.c.)

run slowly into the hypobromite solution. When bubbles have ceased to rise, read off the height of the fluid in the large tube by the graduations upon its side. This gives the amount by weight of urea in the urine added, from which the amount excreted in twenty-four hours can easily be calculated. If the urine contains much more than the normal amount, it should be diluted.

To avoid handling pure bromin, which is disagreeable, Rice's solutions may be employed:

(*a*) Bromin,	31 gm.	
Potassium bromid,	31 "	
Distilled water,	250 c.c.	
(*b*) Caustic soda,	100 gm.	
Distilled water,	250 c.c.	

One part of each of these solutions and two parts of water are mixed and used for the test. The bromin solution must be kept in a tightly stoppered bottle or it will rapidly lose strength.

5. Uric Acid.—Uric acid is the most important of a group of substances, called *purin bodies*, which are derived chiefly from the nucleins of the food and from metabolic destruction of the nuclei of the body. The daily output of uric acid is about 0.4 to 1 gm. The amount of the other purin bodies together is about one-tenth that of uric acid. Excretion of these substances is greatly increased by a diet rich in nucleins, as sweetbreads and liver.

Uric acid exists in the urine in the form of urates, which in concentrated urines are readily thrown out of solution and constitute the familiar sediment of " amorphous urates." This, together with the fact that uric
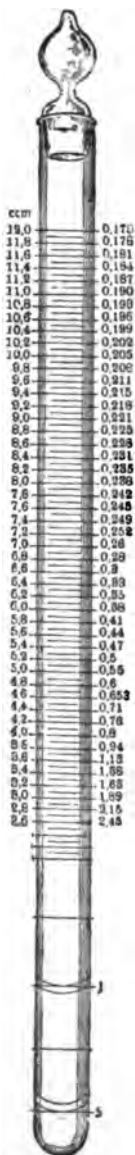
Fig. 25.—Ruhemann's uricometer.

acid is frequently deposited as crystals, constitutes its chief interest to the practitioner. It is a very common error to consider these deposits as evidence of excessive excretion.

Pathologically, the greatest increase of uric acid occurs in leukemia, where there is extensive destruction of leukocytes, and in diseases with active destruction of the liver and other organs rich in nuclei. Uric acid is decreased before an attack of gout and increased during it, but its etiologic relation is still uncertain. An increase is also noted in acute articular rheumatism during the febrile stage.

Quantitative Estimation.—The following are the best methods for ordinary clinical purposes, although no great accuracy can be claimed for them.

Cook's Method for Purin Bodies.—In a centrifuge tube take 10 c.c. urine and add about 1 gm. (about 1 c.c.) sodium carbonate and 1 or 2 c.c. strong ammonia. Shake until the soda is dissolved. The earthy phosphates will be precipitated. Centrifugalize thoroughly and pour off all the clear fluid into a graduated centrifuge tube. Add 2 c.c. ammonia and 2 c.c. ammoniated silver nitrate solution. Let stand a few minutes, and revolve in the centrifuge until the bulk of precipitate *remains constant.* Each one-tenth cubic centimeter

of sediment represents 0.001176 gm. purin bodies. This amount may be regarded as uric acid, since this substance usually constitutes nine-tenths of the purin bodies and the clinical significance is the same.

Ammoniated silver nitrate solution is prepared by dissolving 5 gm. of silver nitrate in 100 c.c. distilled water, and adding ammonia until the solution clouds and again becomes clear.

Ruhemann's Method for Uric Acid.—The urine must be slightly acid. Fill Ruhemann's tube (Fig. 25) to the mark *S* with the indicator, carbon disulphid, and to the mark *J* with the reagent. The carbon disulphid will assume a violet color. Add the urine, a small quantity at a time, closing the tube with the glass stopper and shaking vigorously after each addition, until the disulphid loses every trace of its violet color and becomes pure white. This completes the test. The figure in the right-hand column of figures corresponding to the top of the fluid gives the amount of uric acid in parts per thousand. The presence of diacetic acid interferes with the test, as do also, to some extent, bile and albumin.

Ruhemann's reagent consists of iodin and potassium iodid each, 1.5 parts; absolute alcohol, 15 parts; and distilled water, 185 parts.

6. Ammonia.—A small amount of ammonia, combined with hydrochloric, phosphoric, and sulphuric acids is always present. Estimated as NH_3, the normal average is about 0.7 gm. in twenty-four hours. This represents 4 to 5 per cent. of the total nitrogen of the urine, ammonia standing next to urea in this respect.

Under ordinary conditions, most of the ammonia which results from the metabolic processes is transformed into urea. When, however, acids are present in excess, either from ingestion of mineral acids or

7

from abnormal production of acids within the body (as in fevers, diabetes, pernicious vomiting of pregnancy, etc.), ammonia combines with them and is so excreted, urea being correspondingly decreased. It is thus that the body protects itself against acid intoxication. A marked increase of ammonia is, therefore, important chiefly as an index of the tendency to acidosis, particularly that associated with the presence of diacetic and oxybutyric acids.

In diabetes mellitus ammonia elimination may reach 4 or 5 gm. daily. It is likewise markedly increased in pernicious vomiting of pregnancy, but *not in nervous vomiting;* and in conditions in which the power to synthesize urea is interfered with, notably cirrhosis and other destructive diseases of the liver and conditions associated with deficient oxygenation.

Quantitative Estimation.—The urine must be fresh, since decomposition increases the amount of ammonia. The following method is satisfactory for clinical purposes, though subject to some inaccuracies.

Ronchese-Malfatti Formalin Test.—This depends upon the fact that when formalin is added to the urine, the ammonia combines with it, forming hexamethylene-tetramin. The acids with which the ammonia was combined are set free, and their quantity, ascertained by titration with sodium hydroxid, indicates the amount of ammonia.

Take 10 c.c. of the urine in a beaker or evaporating dish, add 50 c.c. water and 10 drops of 0.5 per cent. alcoholic solution of phenolphthalein. Neutralize by adding a weak caustic soda or sodium carbonate solution until a permanent pink color appears. To 5 c.c. formalin add 15 c.c. water and neutralize in the same way. Pour the formalin into the

urine. The pink color at once disappears, owing to liberation of acids. Now add decinormal sodium hydroxid solution from a buret until the pink color just returns. Each cubic centimeter of the decinormal solution used in this titration corresponds to 0.0017 gm. of NH_3. This must be multiplied by ten to obtain the percentage from which the twenty-four-hour elimination of ammonia is calculated.

The method is more complicated, but distinctly more accurate when carried out as suggested by E. W. Brown. Treat 60 c.c. of urine with 3 gm. of basic lead acetate, stir well, let stand a few minutes, and filter. Treat the filtrate with 2 gm. neutral potassium oxalate, stir well, and filter. Take 10 c.c. of the filtrate, add 50 c.c. water and 15 gm. neutral potassium oxalate, and proceed with the ammonia estimation as above outlined.

B. ABNORMAL CONSTITUENTS

Those substances which appear in the urine only in pathologic conditions are of much more interest to the clinician than are those which have just been discussed. Among them are: proteins, sugars, the acetone bodies, bile, hemoglobin, and the diazo substances. The " pancreatic reaction " and detection of drugs in the urine will also be discussed under this head.

1. Proteins.—Of the proteins which may appear in the urine, serum-albumin and serum-globulin are the most important. Mucin, proteose, and a few others are found occasionally, but are of less interest.

(1) **Serum-albumin and Serum-globulin.**—These two proteins constitute the so-called " urinary albumin." They usually occur together, have practically the same significance, and both respond to all the ordinary tests for " albumin."

Their presence, or *albuminuria*, is probably the most important pathologic condition of the urine. It is either *accidental* or *renal*. The physician can make no greater mistake than to regard all cases of albuminuria as indicating kidney disease.

Accidental or *false albuminuria* is due to admixture with the urine of albuminous fluids, such as pus, blood, and vaginal discharge. The microscope will usually reveal its nature. It occurs most frequently in pyelitis, cystitis, and chronic vaginitis.

Renal albuminuria refers to albumin which has passed from the blood into the urine through the walls of the kidney tubules or the glomeruli.

Albuminuria sufficient to be recognized by clinical methods probably never occurs as a physiologic condition, the so-called *physiologic albuminuria* appearing only under conditions which must be regarded as abnormal. Among these may be mentioned excessive muscular exertion in those unaccustomed to it; excessive ingestion of proteins; prolonged cold baths; and childbirth. In these conditions the albuminuria is slight and transient.

There are certain other forms of albuminuria which have still less claim to be called physiologic, but which are not always regarded as pathologic. Among these are *cyclic albuminuria*, which regularly recurs at a certain period of the day, and *orthostatic* or *postural albuminuria*, which appears only when the patient is standing. They are rare and of obscure origin, and occur for the most part in neurasthenic subjects during adolescence. It is noteworthy in this connection that nephritis sometimes begins with a cyclic albuminuria.

In pathologic conditions and in most, at least, of the "functional" conditions just enumerated, renal albuminuria may be referred to one or more of the following causes. In nearly all cases it is accompanied by tube-casts.

(a) *Changes in the blood* which render its albumin more diffusible, as in severe anemias, purpura, and scurvy. Here the albumin is small in amount.

(b) *Changes in circulation in the kidney*, either anemia or congestion, as in excessive exercise, chronic heart disease, and pressure upon the renal veins. The quantity of albumin is usually, but not always, small. Its presence is constant or temporary, according to the cause. Most of the causes, if continued, will produce organic changes in the kidney.

(c) *Organic Changes in the Kidney.*—These include the inflammatory and degenerative changes commonly grouped together under the name of nephritis, and also renal tuberculosis, neoplasms, and cloudy swelling due to irritation of toxins and drugs. The amount of albumin eliminated in these conditions varies from minute traces to 20 gm., or even more, in the twenty-four hours, and, except in acute processes, bears little relation to the severity of the disease. In acute and chronic parenchymatous nephritis the quantity is usually very large. In chronic interstitial nephritis it is small— frequently no more than a trace. It is small in cloudy swelling from toxins and drugs, and variable in renal tuberculosis and neoplasms. In amyloid disease of the kidney the quantity is usually small, and serum-globulin may be present in especially large proportion, or even alone. Roughly distinctive of serum-globulin is the

appearance of an opalescent cloud when a few drops of the urine are dropped into a glass of distilled water.

Detection of albumin depends upon its precipitation by chemicals or coagulation by heat. There are many tests, but none is entirely satisfactory, because other substances as well as albumin are precipitated. The most common source of error is mucin. The tests given here are widely used and can be recommended. They make no distinction between serum-albumin and serum-globulin. They are given as nearly as possible in order of their delicacy. Usually the best time to detect albumin is in the evening or a few hours after a meal.

It is very important that urine to be tested for albumin be rendered clear by filtration or centrifugation. This is too often neglected in routine work. When ordinary methods do not suffice, it can usually be cleared by shaking up with a little purified talc or animal charcoal and filtering.

(1) **Trichloracetic Acid Test.**—The reagent consists of a saturated aqueous solution of trichloracetic acid to which magnesium sulphate is added to saturation. A simple saturated solution of the acid may be used, but addition of magnesium sulphate favors precipitation of globulin, and, by raising the specific gravity, makes the test easier to apply.

Take a few cubic centimeters of the reagent in a test-tube or conical test glass, hold the tube or glass in an inclined position, and run the urine gently in by means of a pipet, so that it will form a layer on top of the reagent without mixing with it. If albumin be present, a white, cloudy ring will appear where the two fluids come in contact. The ring can be seen most clearly if viewed against a black background,

and one side of the tube or conical glass may be painted black for this purpose.

This is an extremely sensitive test, but, unfortunately, both mucin and proteoses respond to it; urates, when abundant, may give a confusing white ring, and the reagent is comparatively expensive. It is not much used in routine work except as a control to the less sensitive tests.

Fig. 26.—Horismascope: adding the reagent.

A most convenient instrument for applying this or any of the contact tests is sold under the name of " horismascope " (Fig. 26).

(2) **Robert's Test.**—The reagent consists of pure nitric acid, 1 part, and saturated aqueous solution of magnesium sulphate, 5 parts. It is applied in the same way as the preceding test.

Albumin gives a white ring, which varies in density with

the amount present. A similar white ring may be produced by primary proteose and resinous drugs. White rings or cloudiness in the urine above the zone of contact may result from excess of urates or mucus. Colored rings near the junction of the fluids may be produced by urinary pigments, bile, or indican.

Robert's test is one of the best for routine work, although the various rings are apt to be confusing to the inexperienced. It is more sensitive than Heller's test, of which it is a modification, and has the additional advantage that the reagent is not so corrosive.

(3) **Purdy's Heat Test.**—Take a test-tube two-thirds full of urine, add about one-sixth its volume of saturated solution of sodium chlorid, and 5 to 10 drops of 50 per cent. acetic acid. Mix, and boil the upper inch. A white cloud in the heated portion shows the presence of albumin.

This is a valuable test for routine work. It is simple, sufficiently accurate for clinical purposes, and has practically no fallacies. *Addition of the salt solution, by raising the specific gravity, prevents precipitation of mucin.* Proteose may produce a white cloud, which disappears upon boiling and reappears upon cooling.

(4) **Heat and Nitric Acid Test.**—This is one of the oldest of the albumin tests, and if properly carried out, one of the best. Boil a small quantity of filtered urine in a test-tube and add about one-twentieth its volume of concentrated nitric acid. A white cloud or flocculent precipitate (which usually appears during the boiling, but if the quantity be very small only after addition of the acid) denotes the presence of albumin. A similar white precipitate, which disappears upon addition of the acid, is due to earthy phosphates. The acid should not be added before boiling, and the proper amount should always be used; otherwise, part of the albumin may fail to be precipitated or may be redissolved.

Quantitative Estimation.—The gravimetric, which is the most reliable method, is too elaborate for clinical work. Both Esbach's, which is very widely used, and the centrifugal method give fair results, but Tsuchiya's recent modification of the Esbach method is preferable to either.

(1) **Esbach's Method.**—The urine must be clear, of acid reaction, and not concentrated. Always filter before testing, and, if necessary, add acetic acid and dilute with water. Esbach's tube (Fig. 27) is essentially a test-tube with a mark U near the middle, a mark R near the top, and graduations $\frac{1}{2}$, 1, 2, 3, etc., near the bottom. Fill the tube to the mark U with urine and to the mark R with the reagent. Close with a rubber stopper, invert slowly several times, and set aside in a cool place. At the end of twenty-four hours read off the height of the precipitate. This gives the amount of albumin in *grams per liter, and must be divided by 10 to obtain the percentage.*

Fig. 27.—Esbach's albuminometer, improved form.

Esbach's reagent consists of picric acid, 1 gm., citric acid, 2 gm., and distilled water, to make 100 c.c.

(2) **Tsuchiya's Method.**—This is carried out in the same manner as the Esbach method, using the following reagent:

Phosphotungstic acid.............. 1.5 gm.
96 per cent. alcohol................ 95.0 c.c.
Concentrated hydrochloric acid...... 5.0 "

The urine should be diluted to a specific gravity not exceeding 1.008. The method is said to be much more accurate than

the original Esbach method, particularly with small quantities of albumin.

(3) **Purdy's Centrifugal Method.**—This is detailed in the table on opposite page. The percentage by weight is approximately one-fiftieth of the bulk percentage.

(2) **Mucin.**—Traces of the substances (mucin, mucoid, etc.) which are loosely classed under this name are present in normal urine; increased amounts are observed in irritations and inflammations of the mucous membrane of the urinary tract. They are of interest chiefly because they may be mistaken for albumin in most of the tests. If the urine be diluted with water and acidified with acetic acid, the appearance of a white cloud indicates the presence of mucin.

True mucin is a glyco-protein, and upon boiling with an acid or alkali, as in Fehling's test, yields a carbohydrate substance which reduces copper.

(3) **Proteoses.**—These are intermediate products in the digestion of proteins and are frequently, although incorrectly, called albumoses. Two groups are generally recognized: *primary proteoses*, which are precipitated upon half-saturation of their solutions with ammonium sulphate; and *secondary proteoses*, which are precipitated only upon complete saturation.

The secondary proteoses have been observed in the urine in febrile and malignant diseases and chronic suppurations, during resolution of pneumonia, and in many other conditions, but their clinical significance is indefinite. In pregnancy, albumosuria may be due to absorption of amniotic fluid.

Primary proteoses are rarely encountered in the urine.

PURDY'S QUANTITATIVE METHOD FOR ALBUMIN IN URINE (CENTRIFUGAL).

Table showing the relation between the volumetric and gravimetric percentage of albumin obtained by means of the centrifuge with radius of six and three-quarter inches; rate of speed, 1500 revolutions per minute; time, three minutes.

VOLUMETRIC PERCENTAGE BY CENTRIFUGE.	PERCENTAGE BY WEIGHT OF DRY ALBUMIN.	GRAINS PER FLUIDOUNCE DRY ALBUMIN.	VOLUMETRIC PERCENTAGE BY CENTRIFUGE.	PERCENTAGE BY WEIGHT OF DRY ALBUMIN.	GRAINS PER FLUIDOUNCE DRY ALBUMIN.	VOLUMETRIC PERCENTAGE BY CENTRIFUGE.	PERCENTAGE BY WEIGHT OF DRY ALBUMIN.	GRAINS PER FLUIDOUNCE DRY ALBUMIN.
¼	0.005	0.025	13½	0.281	1.35	31½	0.656	3.15
½	0.01	0.05	14	0.292	1.4	32	0.667	3.2
¾	0.016	0.075	14½	0.302	1 45	32½	0.677	3.25
1	0.021	0.1	15	0.313	1.5	33	0.687	3.3
1¼	0.026	0.125	15¼	0.323	1.55	33½	0.698	3.35
1½	0.031	0.15	16	0.333	1.6	34	0.708	3.4
1¾	0.036	0.175	16½	0.344	1.65	34½	0.719	3.45
2	0.042	0.2	17	0.354	1.7	35	0.729	3.5
2¼	0.047	0.225	17½	0.365	1.75	35½	0.74	3.55
2½	0.052	0.25	18	0.375	1.8	36	0.75	3.6
2¾	0.057	0.275	18½	0.385	1.85	36½	0.76	3.65
3	0.063	0.3	19	0.396	1.9	37	0.771	3.7
3¼	0.068	0.325	19½	0.406	1.95	37½	0.781	3.75
3½	0.073	0.35	20	0.417	2.	38	0.792	3.8
3¾	0.078	0.375	20½	0.427	2.05	38½	0.801	3.85
4	0.083	0.4	21	0.438	2.1	39	0.813	3.9
4¼	0.089	0.425	21½	0.448	2.15	39½	0.823	3.95
4½	0.094	0.45	22	0.458	2.2	40	0.833	4.
4¾	0.099	0.475	22½	0.469	2.25	40½	0.844	4.05
5	0.104	0.5	23	0.479	2.3	41	0.854	4.1
5½	0.111	0.55	23½	0.49	2.35	41½	0.865	4.15
6	0.125	0.6	24	0.5	2.4	42	0.875	4.2
6½	0.135	0.65	24½	0.51	2.45	42½	0.885	4.25
7	0.146	0.7	25	0.521	2.5	43	0.896	4.3
7½	0.156	0.75	25½	0.531	2.55	43½	0.906	4.35
8	0.167	0.8	26	0.542	2.6	44	0.917	4.4
8½	0.177	0.85	26½	0.552	2.65	44½	0.927	4.45
9	0.187	0.9	27	0.563	2.7	45	0.938	4.5
9½	0.198	0.95	27½	0.573	2.75	45½	0.948	4.55
10	0.208	1.	28	0.583	2.8	46	0.958	4.6
10½	0.219	1.05	28½	0.594	2.85	46½	0.969	4.65
11	0.229	1.1	29	0.604	2.9	47	0.979	4.7
11½	0.24	1.15	29½	0.615	2.95	47½	0.99	4.75
12	0.25	1.2	30	0.625	3.	48	1.	4.8
12½	0.26	1.25	30½	0.635	3.05	...	...	...
13	0.271	1.3	31	0.646	3.1	...	...	...

Test.—Three cubic centimeters of 10 per cent. solution of ferrocyanid of potassium and 2 cubic centimeters of 50 per cent. acetic acid are added to 10 cubic centimeters of the urine in the percentage tube and *stood aside for ten minutes*, then placed in the centrifuge and revolved at rate of speed and time as stated at head of the table. If albumin is excessive, dilute the urine with water until volume of albumin falls below 10 per cent. Multiply result by the number of dilutions employed before using the table.

The protein known as the " Bence-Jones body " was originally classed under this head, but its true nature is uncertain. It is regarded as practically pathognomonic of multiple myeloma.

The proteoses are not coagulable by heat, but are precipitated by such substances as trichloracetic acid and phosphotungstic acid. The primary proteoses, alone, are precipitated by nitric acid.

Proteoses may be detected by acidifying the urine with acetic acid, boiling and filtering while hot to remove mucin, albumin, and globulin, and testing the filtrate by the trichloracetic acid test. As above indicated, the nitric acid test, and half and complete saturation with ammonium sulphate will separate the two groups.

To detect Bence-Jones' body the urine is acidified with acetic acid and gently heated. If this substance be present, a precipitate will form at about 60° C. As the boiling-point is reached, it wholly or partially dissolves. It reappears upon cooling.

2. Sugars.—Various sugars may at times be found in the urine. Dextrose is by far the most common, and is the only one of clinical importance. Levulose, lactose, and some others are occasionally met with.

(1) **Dextrose (Glucose).**—It is probable that traces of glucose, too small to respond to the ordinary tests, are present in the urine in health. Its presence in appreciable amount constitutes " glycosuria."

Transitory glycosuria is unimportant, and may occur in many conditions, as after general anesthesia and administration of certain drugs, in pregnancy, and following shock and head injuries. It may also occur

after eating excessive amounts of carbohydrates (alimentary glycosuria).

Persistent glycosuria has been noted in brain injuries involving the floor of the fourth ventricle. As a rule, however, persistent glycosuria is diagnostic of diabetes mellitus, of which disease it is the essential symptom. The amount of glucose eliminated in diabetes is usually considerable, and is sometimes very large, reaching 500 gm., or even more, in twenty-four hours, but it does not bear any uniform relation to the severity of the disease. Glucose may, on the other hand, be almost or entirely absent temporarily.

Detection of Dextrose.—If albumin be present in more than traces, it must be removed by boiling and filtering.

(1) **Haines' Test.**—Take about 1 dram of Haines' solution in a test-tube, boil, and add 6 or 8 drops of urine. A heavy yellow or red precipitate, which settles readily to the bottom, shows the presence of sugar. Neither precipitation of phosphates as a light, flocculent sediment nor simple decolorization of the reagent should be mistaken for a positive reaction.

This is one of the best of the copper tests, all of which depend upon the fact that in strongly alkaline solutions glucose reduces cupric hydrate to cuprous hydrate (yellow) or cuprous oxid (red). They are somewhat inaccurate, because they make no distinction between glucose and less common forms of sugar; because certain normal substances, when present in excess, especially mucin, uric acid, and creatinin, may reduce copper, and because many drugs—*e. g.*, chloral, chloroform, copaiba, acetanilid, benzoic acid, morphin, sulphonal, salicylates—are eliminated as copper-reducing substances. To minimize these fallacies *dilute the urine, if it be concentrated; do not add more than the specified amount of urine, and do not boil after the urine is added.*

Haines' solution is prepared as follows: completely dissolve 30 gr. pure copper sulphate in ½ oz. distilled water, and add ½ oz. pure glycerin; mix thoroughly, and add 5 oz. liquor potassæ. The solution keeps well.

(2) **Fehling's Test.**—Two solutions are required—one containing 34.64 gm. pure crystalline copper sulphate in 500 c.c. distilled water; the other, 173 gm. Rochelle salt and 100 gm. potassium hydroxid in 500 c.c. distilled water. Mix equal parts of the two solutions in a test-tube, dilute with
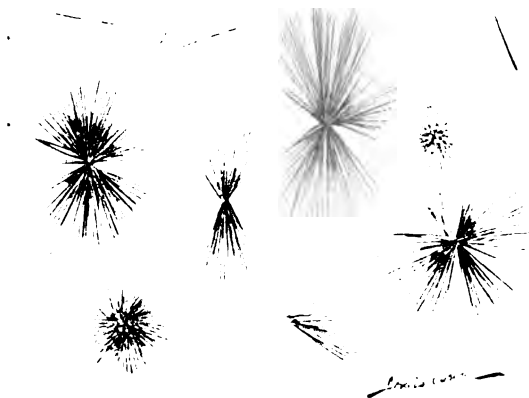
Fig. 28.—Crystals of phenylglucosazone from diabetic urine—Kowarsky's test ($\times$ 500).

3 or 4 volumes of water, and boil. Add the urine a little at a time, heating, but not boiling, between additions. In the presence of glucose a heavy red or yellow precipitate will appear. The quantity of urine should not exceed that of the reagent.

(3) **Benedict's Test.**—This new test promises to displace all other reduction tests for glucose. The reagent is said to be ten times as sensitive as Haines' or Fehling's, and not to be reduced by uric acid, creatinin, chloroform, or the aldehyds. It consists of:

Copper sulphate (pure crystallized),   17.3 gm.
Sodium or potassium citrate,   173.0   "
Sodium carbonate (crystallized),   200.0   "
    (or 100 gm. of the anhydrous salt).
Distilled water, to make   1000.0 c.c.

Dissolve the citrate and carbonate in 700 c.c. of water, with the aid of heat, and filter. Dissolve the copper in 100 c.c. of water and pour slowly into the first solution, stirring constantly. Cool, and make up to one liter. The reagent keeps indefinitely.

Take about 5 c.c. of this reagent in a test-tube, and add *8 or 10 drops (not more)* of the urine. Heat to vigorous boiling, keep at this temperature for one or two minutes, and allow to cool slowly. In the presence of glucose the entire body of the solution will be filled with a precipitate, which may be red, yellow, or green in color. When traces only of glucose are present, the precipitate may appear only upon cooling. In the absence of glucose, the solution remains clear or shows only a faint, *bluish* precipitate, due to urates.

(4) **Phenylhydrazin Test.**— *Kowarsky's Method.*—In a wide test-tube take 5 drops pure phenylhydrazin, 10 drops glacial acetic acid, and 1 c.c. saturated solution of sodium chlorid. A curdy mass results. Add 2 or 3 c.c. urine, boil for at least two minutes, and set aside to cool. Examine the sediment with the microscope, using a two-thirds objective. If glucose be present, characteristic crystals of phenylglucosazone will be seen. These are yellow, needle-like crystals arranged mostly in clusters or in sheaves (Fig. 28). When traces only of glucose are present, the crystals may not appear for one-half hour or more. Best crystals are obtained when the fluid is cooled very slowly. It must not be agitated during cooling.

This is an excellent test for clinical work. It requires slightly more time than Haines' test, but more than compensates for this by increased accuracy. It is fully as sensitive as Haines', and has practically no fallacies excepting levulose, which is a fallacy for all tests but the polariscope. Other carbohydrates which are capable of forming crystals with phenylhydrazin are extremely unlikely to do so when the test is applied directly to the urine by the method just detailed. Even if not used routinely, this test should always be resorted to when Haines' test gives a positive reaction in doubtful cases.

Quantitative Estimation.—In quantitative work Fehling's solution, for so many years the standard, has been largely displaced by Purdy's, which avoids the heavy precipitate that so greatly obscures the end-reaction in Fehling's method. The older method is still preferred by many, and both are, therefore, given. The new method of Benedict is likewise included, since it appears to be more exact than any other titration method available for sugar work. Should the urine contain much glucose, it must be diluted before making any quantitative test, allowance being made for the dilution in the subsequent calculation. Albumin, if present, must be removed by acidifying a considerable quantity of urine with acetic acid, boiling, and filtering. The precipitate should then be washed with water and the washings added to the urine to bring it to its original volume.

(1) **Purdy's Method.**—Take exactly 35 c.c. of Purdy's solution in a flask or beaker, add twice its volume of distilled water, heat to boiling, and, still keeping the solution hot, add

the urine very slowly from a buret until the blue color entirely disappears. Read off the amount of urine added; considering the strength of Purdy's solution, it is readily seen that this amount of urine contains 0.02 gm. of glucose, from which the amount in the twenty-four-hour urine, or the percentage, can easily be calculated. Example: Suppose that 2.5 c.c. of urine discharged the blue color of 35 c.c. of Purdy's solution. This amount of urine, therefore, contains exactly 0.02 gm. glucose, and the percentage is obtained from the equation: $2.5 : 100 : : 0.02 : x$, and x equals 0.8 per cent. If, then, the twenty-four-hour quantity of urine were 3000 c.c., the twenty-four-hour elimination of glucose would be found as follows: $100 : 3000 : : 0.8 : x$, and x equals 24 gm.

It will be found that after the test is completed the blue color slowly returns. This is due to reoxidation, and should not be mistaken for incomplete reduction.

A somewhat simpler application of this method, which is accurate enough for clinical purposes, is as follows: Take $8\frac{3}{4}$ c.c. (roughly, 9 c.c.) of Purdy's solution in a large test-tube, dilute with an equal volume of water, heat to boiling, and, while keeping the solution hot but not boiling, add the urine drop by drop from a medicine-dropper until the blue color is entirely gone. Toward the end add the drops very slowly, not more than 4 or 5 a minute. Divide 10 by the number of drops required to discharge the blue color; the quotient will be the percentage of glucose. If 20 drops were required, the percentage would be $10 \div 20 = 0.5$ per cent. It is imperative that the drops be of such size that 20 of them will make 1 c.c. Test the dropper with urine, not water. If the drops are too large, draw out the tip of the dropper; if too small, file off the tip.

Purdy's solution consists of pure crystalline copper sulphate, 4.752 gm.; potassium hydroxid, 23.5 gm.; ammonia (U. S. P.; sp. gr., 0.9), 350 c.c.; glycerin, 38 c.c.; distilled water, to make

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1000 c.c. Dissolve the copper sulphate and glycerin in 200 c.c. of the water by aid of gentle heat. In another 200 c.c. of water dissolve the potassium hydroxid. Mix the two solutions, and when cool, add the ammonia. Lastly, bring the whole up to 1000 c.c. with distilled water. This solution is of such strength that the copper in 35 c.c. will be reduced by exactly 0.02 gm. of glucose.

(2) **Fehling's Method.**—Take 10 c.c. Fehling's solution (made by mixing 5 c.c. each of the copper and alkaline solutions described on page 110) in a flask or beaker, add three or
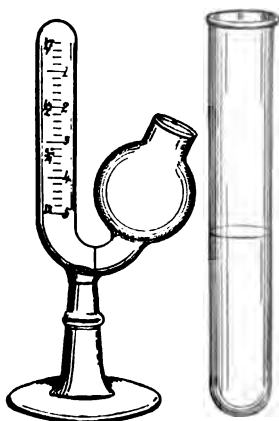
Fig. 29.—Einhorn's saccharimeter.

four volumes of water, boil, and add the urine very slowly from a buret until the solution is completely decolorized, heating but not boiling after each addition.

The chief objection to Fehling's method is the difficulty of determining the end-point. The use of an " outside indicator," however, obviates this. When reduction is thought to be complete, a few drops of the solution are filtered through a fine-grained filter-paper on to a porcelain plate, quickly acidified with acetic acid, and mixed with a drop of 10 per

cent. potassium ferrocyanid. Immediate appearance of a red-brown color shows the presence of unreduced copper.

Fehling's solution is of such strength that the copper in 10 c.c. will be reduced by exactly 0.05 gm. of glucose. Therefore, the amount of urine required to decolorize the test solution contains just 0.05 gm. glucose, and the percentage is easily calculated.

(3) **Benedict's Method.**—The following modification of his copper solution has recently been offered by Benedict for quantitative estimations.

The reagent consists of:

Copper sulphate (pure crystallized),	18.0 gm.
Sodium carbonate (crystallized),	200.0 "
(or 100 gm. of the anhydrous salt).	
Sodium or potassium citrate,	200.0 "
Potassium sulphocyanate,	125.0 "
5 per cent. potassium ferrocyanid solution,	5.0 c.c.
Distilled water, to make	1000.0 "

With the aid of heat dissolve the carbonate, citrate, and sulphocyanate in about 800 c.c. of the water and filter. Dissolve the copper in 100 c.c. of water and pour slowly into the other fluid, stirring constantly. Add the ferrocyanid solution, cool, and dilute to 1000 c.c. Only the copper need be accurately weighed. This solution is of such strength that 25 c.c. are reduced by 0.05 gram glucose. It keeps well.

To make a sugar estimation, take 25 c.c. of the reagent in a porcelain evaporating dish, add 10 to 20 grams sodium carbonate crystals (or one-half this weight of the anhydrous salt) and a small quantity of powdered pumice-stone or talcum. Heat to boiling, and add the urine rather rapidly from a buret until a chalk-white precipitate forms and the blue color of the reagent begins to fade. After this

point is reached, add the urine a few drops at a time until the last trace of blue just disappears. This end-point is easily recognized. During the whole of the titration the mixture must be kept vigorously boiling. Loss by evaporation must be made up by adding water. The quantity of urine required to discharge the blue color contains exactly 0.05 gram glucose, and the percentage contained in the original sample is easily calculated.

(4) **Fermentation Method.**—This is convenient and satisfactory, its chief disadvantage begin the time required. It depends upon the fact that glucose is fermented by yeast with evolution of CO_2. The amount of gas evolved is an index of the amount of glucose. Einhorn's saccharimeter (Fig. 29) is the simplest apparatus.

The urine must be so diluted as to contain not more than 1 per cent. of glucose. A fragment of fresh yeast cake about the size of a split-pea is mixed with a definite quantity of the urine measured in the tube which accompanies the apparatus. It should form an emulsion free from lumps or air-bubbles. The long arm of the apparatus is then filled with the mixture. At the end of fifteen to twenty-four hours fermentation will be complete, and the percentage of glucose can be read off upon the side of the tube. The result must then be multiplied by the degree of dilution. Since yeast itself sometimes gives off gas, a control test must be carried out with normal urine and the amount of gas evolved must be subtracted from that of the test. A control should also be made with a known glucose solution to make sure that the yeast is active.

(5) **Robert's Differential Density Method.**—While this method gives only approximate results, it is convenient, and requires no special apparatus but an accurate urinometer. Mix a quarter of a yeast-cake with about 4 oz. of urine. Take the specific gravity and record it. Set the urine in a warm place for twenty-four hours or until fermentation is com-

plete. Then cool to the temperature at which the specific gravity was originally taken, and take it again. The difference between the two readings gives the number of grains of sugar per ounce, and this, multiplied by 0.234, gives the *percentage* of sugar. If the original reading is 1.035, and that after fermentation is 1.020, the urine contains $1.035 - 1.020 = 15$ grains of sugar per fluidounce; and the percentage equals $15 \times 0.234 = 3.5$.

(2) **Levulose, or fruit-sugar,** is very rarely present in the urine except in association with glucose, and has about the same significance. Its name is derived from the fact that it rotates polarized light to the left. It behaves the same as glucose with all the ordinary tests, and is not readily distinguished except by polarization.

(3) **Lactose, or milk-sugar,** is sometimes present in the urine of nursing women and in that of women who have recently miscarried. It is of interest chiefly because it may be mistaken for glucose. *It reduces copper, but does not ferment with yeast.* In strong solution it can form crystals with phenylhydrazin, but is extremely unlikely to do so when the test is applied directly to the urine.

(4) **Pentoses.**—These sugars are so named because they contain five atoms of oxygen. Vegetable gums form their chief source. They reduce copper strongly but slowly, and give crystals with phenylhydrazin, but do not ferment with yeast.

Pentosuria is uncommon. It has been noted after ingestion of large quantities of pentose-rich substances, such as cherries, plums, and fruit-juices, and is said to be fairly constant in habitual use of morphin. It sometimes accompanies glycosuria in diabetes. An obscure

chronic form of pentosuria without clinical symptoms has been observed.

Bial's Orcin Test.—Dextrose is first removed by fermentation. About 5 c.c. of Bial's reagent are heated in a test-tube, and after removing from the flame the urine is added drop by drop, not exceeding twenty drops in all. The appearance of a green color denotes pentose.

The reagent consists of:

30 per cent. hydrochloric acid.......	500 c.c.
10 per cent. ferric chlorid solution.....	25 drops
Orcin.............................	1 gram.

3. Acetone Bodies.—This is a group of closely related substances—acetone, diacetic acid, and beta-oxybutyric acid. Acetone is derived from decomposition of diacetic acid, and this in turn from beta-oxybutyric acid by oxidation. The origin of beta-oxybutyric acid is not definitely known, but it is probable that its chief, if not its only, source is in some obscure metabolic disturbance with abnormal destruction of fats. The three substances generally appear in the urine in the order mentioned. When the disturbance is mild, acetone only appears; as it becomes more marked, diacetic acid is added, and finally beta-oxybutyric acid appears. The presence of beta-oxybutyric acid in the blood is probably the chief cause of the form of auto-intoxication known as " acid intoxication."

(1) **Acetone.**—Minute traces, too small for the ordinary tests, may be present in the urine under normal conditions. Larger amounts are not uncommon in fevers, gastro-intestinal disturbances, and certain nervous disorders. A notable degree of acetonuria has

likewise been observed in pernicious vomiting of pregnancy and in eclampsia.

Acetonuria is practically always observed in acid intoxication, and, together with diaceturia, constitutes its most significant diagnostic sign. A similar or identical toxic condition, always accompanied by acetonuria and often fatal, is now recognized as a not infrequent late effect of anesthesia, particularly of chloroform anesthesia. This postanesthetic toxemia is more likely to appear, and is more severe when the urine contains any notable amount of acetone before operation, which suggests the importance of routine examination for acetone in surgical cases.

Acetone is present in considerable amounts in many cases of diabetes mellitus, and is always present in severe cases. Its amount is a better indication of the severity of the disease than is the amount of sugar. A progressive increase is a grave prognostic sign. It can be diminished temporarily by more liberal allowance of carbohydrates in the diet.

According to Folin, acetone is present in only small amounts in these conditions, the substance shown by the usual tests, particularly after distillation of the urine, being really diacetic acid. In this connection, Frommer's test is to be recommended, since it does not require distillation, and does not react to diacetic acid unless too great heat is applied.

Detection of Acetone.—The urine may be tested directly, but it is best to distil it after adding a little phosphoric or hydrochloric acid to prevent foaming, and to test the first few cubic centimeters of distillate. A simple distilling apparatus is shown in Fig. 30. The

test-tube may be attached to the delivery tube by means of a two-hole rubber cork as shown, the second hole serving as air vent, or, what is much less satisfactory, it may be tied in place with a string. Should the vapor not condense well, the test-tube may be immersed in a glass of cold water.

Fig. 30.—A simple distilling apparatus.

When diacetic acid is present, a considerable proportion will be converted into acetone during distillation.

(1) **Gunning's Test.**—To a few cubic centimeters of urine or distillate in a test-tube add a few drops of tincture of iodin and of ammonia alternately until a heavy black cloud appears. This cloud will gradually clear up, and if acetone be present, iodoform, usually crystalline, will separate out. The iodoform can be recognized by its odor, especially upon heating (there

is danger of explosion if the mixture be heated before the black cloud disappears), or by detection of the crystals microscopically. The latter only is safe, unless one has an unusually acute sense of smell. Iodoform crystals are yellowish, six-pointed stars or six-sided plates (Fig. 31).

This modification of Lieben's test is less sensitive than the original, but is sufficient for all clinical work; it has the advantage that alcohol does not cause confusion, and especially that the sediment of iodoform is practically always crystalline. When applied directly to the urine, phosphates are precipi-
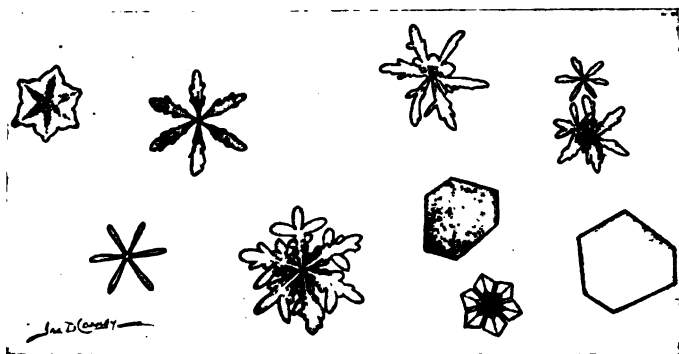
Fig. 31.—Iodoform crystals obtained in several tests for acetone by Gunning's method
(X about 600).

tated and may form star-shaped crystals which are very confusing to the inexperienced. Albumin prevents formation of the crystals, and when it is present, the urine must be distilled for the test.

(2) **Lange's Test.**—This is a modification of the well-known Legal test. It is more sensitive and gives a sharper end-reaction. To a small quantity of urine add about one-twentieth its volume (1 drop for each 1 c.c.) of glacial acetic acid and a few drops of fresh concentrated aqueous solution of sodium nitroprussid, and gently run a little ammonia upon its

surface. If acetone be present, a purple ring will form within a few minutes at the junction of the two fluids.

(3) **Frommer's Test.**—This test has proved very satisfactory in the hands of the writer. The urine need not be distilled. Alkalinize about 10 c.c. of the urine with 2 or 3 c.c. of 40 per cent. caustic soda solution, add 10 or 12 drops of 10 per cent. alcoholic solution of salicylous acid (salicyl aldehyd), heat the upper portion to about 70° C. (it should not reach the boiling-point), and keep at this temperature five minutes or longer. In the presence of acetone an orange color, changing to deep red, appears in the heated portion.

The test can be made more definite by adding the caustic soda in substance (about 1 gram), and before it goes into solution adding the salicyl aldehyd and warming the lower portion.

(2) **Diacetic acid** occurs in the same conditions as acetone, but is less frequent and has more serious significance. In diabetes its presence is a grave symptom and often forewarns of approaching coma. It rarely or never occurs without acetone.

Detection.—The urine must be fresh.

(1) **Gerhardt's Test.**—To a few cubic centimeters of the urine add solution of ferric chlorid (about 10 per cent.) drop by drop until the phosphates are precipitated; filter and add more of the ferric chlorid. If diacetic acid be present, the urine will assume a Bordeaux-red color which disappears upon boiling. A red or violet color which does not disappear upon boiling may be produced by other substances, as phenol, salicylates, and antipyrin.

(2) **Lindemann's Test.**—To about 10 c.c. of urine add 5 drops 30 per cent. acetic acid, 5 drops Lugol's solution, and 2 or 3 c.c. chloroform, and shake. The chloroform does not change color if diacetic acid be present, but becomes reddish

violet in its absence.   This test is claimed by its advocates to
be more sensitive and more reliable than Gerhardt's.

(3) **Oxybutyric acid** has much the same significance
as diacetic acid, but is of more serious import.   There is
no satisfactory clinical test for it.

4. **Bile.**—Bile appears in the urine in all diseases which
produce jaundice, often some days before the skin be-
comes yellow; and in many disorders of the liver not
severe enough to cause jaundice.   It also occurs in dis-
eases with extensive and rapid destruction of red blood-
corpuscles.   Both bile-pigment and bile acids may be
found.   They generally occur together, but the pigment
is not infrequently present alone.   Bilirubin, only, oc-
curs in freshly voided urine, the other pigments (bili-
verdin, bilifuscin, etc.) being produced from this by
oxidation as the urine stands.   The acids are almost
never present without the pigments, and are, therefore,
seldom tested for clinically.

Detection of Bile-pigment.—Bile-pigment gives the
urine a greenish-yellow, yellow, or brown color, which
upon shaking is imparted to the foam.   Cells, casts, and
other structures in the sediment may be stained brown or
yellow.   This, however, should not be accepted as prov-
ing the presence of bile without further tests.

(1) **Smith's Test.**—Overlay the urine with tincture of iodin
diluted with nine times its volume of alcohol.   An emerald-
green ring at the zone of contact shows the presence of bile-
pigments.   It is convenient to use a conical test-glass, one
side of which is painted white.

(2) **Gmelin's Test.**—This consists in bringing slightly
yellow nitric acid into contact with the urine.   A play of

colors, of which green and violet are most distinctive, denotes the presence of bile-pigment. Colorless nitric acid will become yellow upon standing in the sunlight. The test may be applied in various ways: by overlaying the acid with the urine; by bringing a drop of each together upon a porcelain plate; by filtering the urine through thick filter-paper, and touching the paper with a drop of the acid; and, probably best of all, by precipitating with lime-water, filtering, and touching the precipitate with a drop of the acid. In the last method bilirubin is carried down as an insoluble calcium compound.

Detection of Bile Acids.—Hay's test is simple, sensitive, and fairly reliable, and will, therefore, appeal to the practitioner. It depends upon the fact that bile acids lower surface tension. Other tests require isolation of the acids for any degree of accuracy.

Hay's Test.—Upon the surface of the urine, which must not be warm, sprinkle a little finely powdered sulphur. If it sinks at once, bile acids are present to the amount of 0.01 per cent. or more; if only after gentle shaking, 0.0025 per cent. or more. If it remains floating, even after gentle shaking, bile acids are absent.

5. Hemoglobin.—The presence in the urine of hemoglobin or pigments directly derived from it, accompanied by few, if any, red corpuscles, constitutes *hemoglobinuria*. It is a rare condition, and must be distinguished from *hematuria*, or *blood* in the urine, which is common. In both conditions chemic tests will show hemoglobin, but in the latter the microscope will reveal the presence of red corpuscles. Urines which contain notable amounts of hemoglobin have a reddish or brown color, and may deposit a sediment of brown, granular pigment.

Hemoglobinuria occurs when there is such extensive destruction of red blood-cells within the body that the liver cannot transform all the hemoglobin set free into bile-pigment. The most important examples are seen in poisoning, as by mushrooms and potassium chlorate, in scurvy and purpura, in malignant malaria (blackwater fever), and in the obscure condition known as " paroxysmal hemoglobinuria." This last is characterized by the appearance of large quantities of hemoglobin at intervals, usually following exposure to cold, the urine remaining free from hemoglobin between the attacks.

Detection.—Teichmann's test (p. 274) may be applied to the precipitate after boiling and filtering, but the guaiac test is more convenient in routine work.

Guaiac Test.—Mix equal parts of " ozonized " turpentine and fresh tincture of guaiac which has been diluted with alcohol to a light sherry-wine color. In a test-tube or conical glass overlay the urine with this mixture. A bright blue ring will appear at the zone of contact within a few minutes if hemoglobin be present. The guaiac should be kept in an amber-colored bottle. Fresh turpentine can be " ozonized " by allowing it to stand a few days in an open vessel in the sunlight.

This test is very sensitive, and a negative result proves the absence of hemoglobin. Positive results are not conclusive, because numerous other substances—few of them likely to be found in the urine—may produce the blue color. That most likely to cause confusion is pus, but the blue color produced by it disappears upon heating. The thin film of copper often left in a test-tube after testing for sugar may give the reaction, as may also the fumes from an open bottle of bromin.

6. Alkapton Bodies.—The name, alkaptonuria, has been given to a condition in which the urine turns reddish-brown upon standing and strongly reduces copper (but not bismuth), owing to the presence of certain substances which result from imperfect protein metabolism. The change of color takes place quickly when fresh urine is alkalinized, hence the name, *alkapton bodies*.

Alkaptonuria is unaccompanied by other symptoms, and has little clinical importance. Only about forty-five cases, mostly congenital, have been reported. The change in color of the urine and the reduction of copper with no reduction of bismuth nor fermentation with yeast would suggest the condition.

7. Melanin.—Urine which contains melanin likewise darkens upon exposure to the air, assuming a dark brown or black color. This is due to the fact that the substance is eliminated as a chromogen—melanogen—which is later converted into the pigment. ·

Melanuria occurs in most, but not all, cases of melanotic sarcoma. Its diagnostic value is lessened by the fact that it has been observed in other wasting diseases.

Tests for Melanin.—(1) Addition of ferric chlorid gives a gray precipitate which blackens on standing.

(2) Bromin water causes a yellow precipitate which gradually turns black.

8. Diazo Substances.—Certain unknown substances sometimes present in the urine give a characteristic color reaction—the " diazo reaction " of Ehrlich—when treated with diazo-benzol-sulphonic acid and ammonia.

This reaction has much clinical value, provided its limitations be recognized. It is at best an empirical test and must be interpreted in the light of clinical symptoms. Although it has been met with in a considerable number of diseases, its usefulness is practically limited to typhoid fever, tuberculosis, and measles.

(1) **Typhoid Fever.**—Practically all cases give a positive reaction, which varies in intensity with the severity of the disease. It is so constantly present that it is sometimes said to be " negatively pathognomonic ": if negative upon several successive days at a stage of the disease when it should be positive, typhoid is almost certainly absent. Upon the other hand, a reaction when the urine is highly diluted (1: 50 or more) has much positive diagnostic value, since this dilution prevents the reaction in most conditions which might be mistaken for typhoid; but it should be noted that mild cases of typhoid may not give it at this dilution. Ordinarily the diazo appears a little earlier than the Widal reaction,—about the fourth or fifth day,—but it may be delayed. In contrast to the Widal, it begins to fade about the end of the second week, and soon thereafter entirely disappears. An early disappearance is a favorable sign. It reappears during a relapse, and thus helps to distinguish between a relapse and a complication, in which it does not reappear.

(2) **Tuberculosis.**—The diazo reaction has been obtained in many forms of the disease. It has little or no diagnostic value. Its continued presence in pulmonary tuberculosis is, however, a grave prognostic sign, even when the physical signs are slight. After it once appears it generally persists more or less intermit-

tently until death, the average length of life after its appearance being about six months. The reaction is often temporarily present in mild cases during febrile complications, and has then no significance.

(3) **Measles.**—A positive reaction is usually obtained in measles, and may help to distinguish this disease from German measles, in which it does not occur. It generally appears before the eruption and remains about five days.

Technic.—Although the test is really a very simple one, careful attention to technic is imperative. Many of the early workers were very lax in this regard. Faulty technic and failure to record the stage of the disease in which the tests were made have probably been responsible for the bulk of the conflicting results reported.

Certain drugs often given in tuberculosis and typhoid interfere with or prevent the reaction. The chief are creosote, tannic acid and its compounds, opium and its alkaloids, salol, phenol, and the iodids. The reagents are:

(1) Saturated solution sulphanilic acid in 5 per cent. hydrochloric acid.

(2) 0.5 per cent. aqueous solution sodium nitrite.

(3) Strong ammonia.

Mix 100 parts of (1) and one part of (2). In a test-tube take equal parts of this mixture and the urine, and pour 1 or 2 c.c. of the ammonia upon its surface. If the reaction be positive, a garnet ring will form at the junction of the two fluids; and upon shaking, a distinct pink color will be imparted to the foam. The color of the foam is the essential feature. If desired, the mixture may be well shaken before the ammonia is added: the pink color will then instantly appear in that portion of the foam which the ammonia has reached, and can be readily seen. The color varies from eosin-pink to deep

crimson, depending upon the intensity of the reaction. *It is a pure pink or red; any trace of yellow or orange denotes a negative reaction.* A doubtful reaction should be considered negative..

9. Pancreatic Reaction.—Cammidge has shown that in cases of pancreatitis a substance capable of forming crystals with phenylhydrazin can be developed by boiling the urine with a mineral acid, and has offered the following test as an aid in diagnosis of this obscure condition. The nature both of this substance and the antecedent substance from which it is derived is not known. As originally proposed, the test was complicated and probably not trustworthy, but with his improved and simplified technic, Cammidge has had very promising results. In 200 consecutive examinations in which the diagnosis was confirmed, *postmortem* or at operation, 67 cases of pancreatitis (65 chronic, 2 acute) gave positive reactions; 4 cases of cancer of the pancreas were positive, 12 negative; 4 cases in which no pancreatitis was found were positive, 113 were negative. Normal urines do not give the reaction. The difficulty and importance of diagnosis in pancreatitis warrant inclusion of the method here, even though more recent work indicates that its value is not so great as originally claimed.

While the test is somewhat tedious, all the manipulations are simple and require no apparatus but flasks, test-tubes, and funnels.

Technic.—Careful attention to detail is imperative. An ordinary routine examination is first made. Albumin and sugar, if present, must be removed: the former, by acidifying with acetic acid, boiling, and filtering; the latter, by fermenta-

9

tion with yeast after the first step of the method proper. An alkaline urine should be made slightly acid with hydrochloric acid.

(1) Forty cubic centimeters of the urine, which has been rendered perfectly clear by repeated filtration through the same filter-paper are placed in a small flask, treated with 1 c.c. concentrated hydrochloric acid and gently boiled on a

Fig. 32.—"Pancreatic reaction" flasks fitted with funnel condensers on a sand-bath (Robson and Cammidge).

sand-bath for ten minutes, a funnel with long stem being placed in the neck of the flask to act as a condenser (Fig. 32). After boiling, the urine is cooled in a stream of cold water and brought to its original bulk with distilled water; 8 gm. of lead carbonate are then added to neutralize the acid. The fluid is allowed to stand a few minutes and then filtered through well-moistened fine-grain filter-paper until perfectly clear.

(2) The filtrate is shaken up with 8 gm. powdered tribasic lead acetate and filtered. The excess of lead is then removed by passing hydrogen sulphid gas through the fluid (see page 135) or by shaking well with 4 gm. finely powdered sodium sulphate, heating to boiling, cooling to as low a temperature as possible in a stream of water, and filtering as before until perfectly clear.
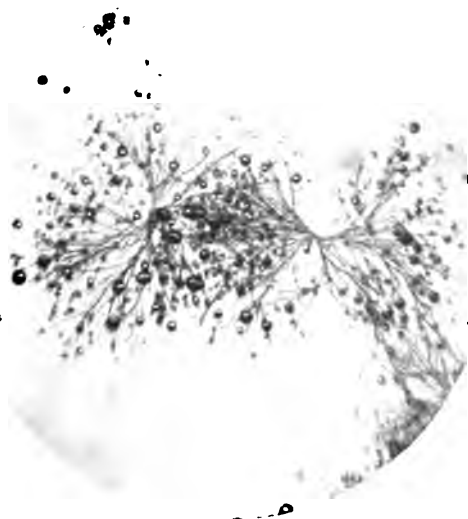
Fig. 33.—Improved "pancreatic reaction." Crystals obtained from a case of chronic pancreatitis with gall-stones in the common duct ($\times$ 200) (from a photo by P. J. Cammidge).

(3) Ten cubic centimeters of the filtrate are then made up to 17 c.c. with distilled water, and added to a mixture of 0.8 gm. phenylhydrazin hydrochlorate, 2 gm. powdered sodium acetate, and 1 c.c. 50 per cent. acetic acid in a small flask with funnel condenser. This is boiled on a sand-bath for ten minutes, and filtered while hot through filter-paper moistened with hot water into a test-tube with a 15 c.c. mark. Should

the filtrate not reach this mark, make up to 15 c.c. with hot distilled water. Allow to cool slowly.

(4) In well-marked cases of pancreatitis a yellow precipitate appears within a few hours; in milder cases, it may not appear for twelve hours. The microscope shows this sediment to consist of " long, light yellow, flexible, hair-like crystals arranged in sheaves, which, when irrigated with 33 per cent. sulphuric acid, melt away and disappear in ten to fifteen seconds after the acid first touches them " (Fig. 33).

(5) To exclude traces of glucose which might be overlooked in the preliminary examination a control test should be carried out in the same manner with omission of step 1.

10. Drugs.—The effect of various drugs upon the color of the urine has been mentioned (p. 71). Most poisons are eliminated in the urine, but their detection is more properly discussed in works upon toxicology. A few drugs which are of interest to the practitioner, and which can be detected by comparatively simple methods, are mentioned here.

Acetanilid and Phenacetin.—The urine is evaporated by gentle heat to about half its volume, boiled for a few minutes with about one-fifth its volume of strong hydrochloric acid, and shaken out with ether. The ether is evaporated, the residue dissolved in water, and the following test applied: To about 10 c.c. are added a few cubic centimeters of 3 per cent. phenol, followed by a weak solution of chromium trioxid (chromic acid) drop by drop. The fluid assumes a red color, which changes to blue when ammonia is added. If the urine is very pale, extraction with ether may be omitted.

Antipyrin.—This drug gives a dark-red color when a few drops of 10 per cent. ferric chlorid are added to the

urine. The color does not disappear upon boiling, which excludes diacetic acid.

Arsenic.—*Reinsch's Test.*—Add to the urine in a test-tube or small flask about one-seventh its volume of hydrochloric acid, introduce a piece of bright copper-foil about one-eighth inch square, and boil for several minutes. . If arsenic be present, a dark-gray film is deposited upon the copper. The test is more delicate if the urine be concentrated by slow evaporation. This test is well known and is widely used, but is not so reliable as the following.

Gutzeit's Test.—In a large test-tube place a little arsenic-free zinc, and add 5 to 10 c.c. pure dilute hydrochloric acid and a few drops of iodin solution (Gram's solution will answer), then add 5 to 10 c.c. of the urine. At once cover the mouth of the tube with a filter-paper cap moistened with saturated aqueous solution of silver nitrate (1:1). If arsenic be present, the paper quickly becomes lemon-yellow, owing to formation of a compound of silver arsenid and silver nitrate, and turns black when touched with a drop of water. To make sure that the reagents are arsenic-free, the paper cap may be applied for a few minutes before the urine is added.

Atropin will cause dilatation of the pupil when a few drops of the urine are placed in the eye of a cat or rabbit.

Bromids can be detected by acidifying about 10 c.c. of the urine with dilute sulphuric acid, adding a few drops of fuming nitric acid and a few cubic centimeters of chloroform, and shaking. In the presence of bromin the chloroform, which settles to the bottom, assumes a yellow color.

Iodin from ingestion of iodids or absorption from

iodoform dressings is tested for in the same way as the bromids, the chloroform assuming a pink to reddish-violet color. To detect traces, a large quantity of urine should be rendered alkaline with sodium carbonate and greatly concentrated by evaporation before testing.

Lead.—No simple method is sufficiently sensitive to detect the traces of lead which occur in the urine in chronic poisoning. Of the more sensitive methods, that of Arthur Lederer is probably best suited to the practitioner:

It is essential that all apparatus used be lead-free. Five hundred cubic centimeters of the urine are acidified with 70 c.c. pure sulphuric acid, and heated in a beaker or porcelain dish. About 20 to 25 gm. of potassium persulphate are added a little at a time. This should decolorize the urine, leaving it only slightly yellow. If it darkens upon heating, a few more crystals of potassium persulphate are added, the burner being first removed to prevent boiling over; if it becomes cloudy, a small amount of sulphuric acid is added. It is then boiled until it has evaporated to 250 c.c. or less. After cooling, an equal volume of alcohol is added, and the mixture allowed to stand in a cool place for four or five hours, during which time all the lead will be precipitated as insoluble sulphate.

The mixture is then filtered through a small, close-grained filter-paper (preferably an ashless, quantitative filter-paper), and any sediment remaining in the beaker or dish is carefully washed out with alcohol and filtered. A test-tube is placed underneath the funnel; a hole is punched through the tip of the filter with a small glass rod, and all the precipitate (which may be so slight as to be scarcely visible) washed down into the test-tube with a

jet of distilled water from a wash-bottle, using as little water as possible. Ten cubic centimeters will usually suffice. This fluid is then heated, adding crystals of sodium acetate until it becomes perfectly clear. It now contains all the lead of the 500 c.c. urine in the form of lead acetate. It is allowed to cool, and hydrogen sulphid gas is passed through it for about five minutes. The slightest yellowish-brown discoloration indicates the presence of lead. A very slight discoloration can be best seen
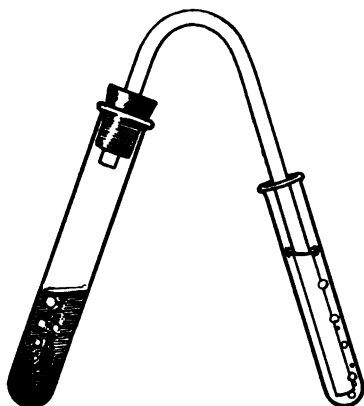
Fig. 34.—A simple hydrogen sulphid generator.

when looked at from above. For comparison, the gas may be passed through a test-tube containing an equal amount of distilled water. The quantity of lead can be determined by comparing the discoloration with that produced by passing the gas through lead acetate (sugar of lead) solutions of known strength. One gram of lead acetate crystals contains 0.54 gram of lead. Hydrogen sulphid is easily prepared in the simple apparatus shown in Fig. 34. A small quantity of iron sulphid is placed

in the test-tube; a little dilute hydrochloric acid is added; the cork is replaced; and the delivery tube is inserted to the bottom of the fluid to be tested.

Mercury.—Traces can be detected in the urine for a considerable time after the use of mercury compounds by ingestion or inunction.

About a liter of urine is acidified with 10 c.c. hydrochloric acid, and a small piece of copper-foil or gauze is introduced. This is gently heated for an hour, and allowed to stand for twenty-four hours. The metal is then removed, and washed successively with very dilute sodium hydroxid solution, alcohol, and ether. When dry, it is placed in a long, slender test-tube, and the lower portion of the tube is heated to redness. If mercury be present, it will volatilize and condense in the upper portion of the tube as small, shining globules which can be seen with a hand-magnifier or low power of the microscope. If, now, a crystal of iodin be dropped into the tube and gently heated, the mercury upon the side of the tube is changed first to the yellow iodid, and later to the red iodid, which are recognized by their color.

Morphin.—Add sufficient ammonia to the urine to render it distinctly ammoniacal, and shake thoroughly with a considerable quantity of pure acetic ether. Separate the ether and evaporate to dryness. To a little of the residue in a watch-glass or porcelain dish add a few drops of formaldehyd-sulphuric acid, which has been freshly prepared by adding one drop of formalin to 1 c.c. pure concentrated sulphuric acid. If morphin be present, this will produce a purple-red color, which changes to violet, blue-violet, and finally nearly pure blue.

Phenol.—As has been stated, the urine following

phenol poisoning turns olive-green and then brownish-black upon standing. Tests are of value in recognizing poisoning from ingestion and in detecting absorption from carbolized dressings.

The urine is acidulated with hydrochloric acid and distilled. To the first few cubic centimeters of distillate is added 10 per cent. solution of ferric chlorid drop by drop. The presence of phenol causes a deep amethyst-blue color, as in Uffelmann's test for lactic acid.

Phenolphthalein, which is now widely used as a cathartic, gives a bright pink color when the urine is rendered alkaline with caustic soda.

Quinin.—A considerable quantity of the urine is rendered alkaline with ammonia and extracted with ether; the ether is evaporated, and a portion of the residue dissolved in about twenty drops of dilute alcohol. The alcoholic solution is acidulated with dilute sulphuric acid, a drop of an alcoholic solution of iodin (tincture of iodin diluted about ten times) is added, and the mixture is warmed. Upon cooling, an iodin compound of quinin (herapathite) will separate out in the form of a microcrystalline sediment of green plates.

The remainder of the residue may be dissolved in a little dilute sulphuric acid. This solution will show a characteristic blue fluorescence when quinin is present.

Resinous drugs cause a white precipitate like that of albumin when strong nitric acid is added to the urine. This is dissolved by alcohol.

Salicylates, salol, and similar drugs give a bluish-violet color, which disappears upon heating, upon addition of a few drops of 10 per cent. ferric chlorid solution. When the quantity of salicylates is small, the urine may

be acidified with hydrochloric acid and extracted with ether, the ether evaporated, and the test applied to an aqueous solution of the residue.

Tannin and its compounds appear in the urine as gallic acid, and the urine becomes greenish-black (inky, if much gallic acid be present) when treated with a solution of ferric chlorid.

III. MICROSCOPIC EXAMINATION

A careful microscopic examination will often reveal structures of great diagnostic importance in urine which seems perfectly clear, and from which only very slight sediment can be obtained with the centrifuge. Upon the other hand, cloudy urines with abundant sediment are often shown by the microscope to contain nothing of clinical significance.

Since the nature of the sediment soon changes, the urine must be examined while fresh, preferably within six hours after it is voided. The sediment is best obtained by means of the centrifuge. If a centrifuge is not available, the urine may be allowed to stand in a conical test-glass for six to twenty-four hours after adding some preservative (p. 69). The " torfuge " (Fig. 35) is said to be a very satisfactory substitute for the centrifuge, and is readily portable.

A small amount of the sediment should be transferred to a slide by means of a pipet. It is very important to do this properly. The best pipet is a small glass tube which has been drawn out at one end to a tip with rather small opening. The tube or glass containing the sediment is held on a level with the eye, the larger end of the pipet is closed with the index-finger, which must be dry, and the

tip is carried down into the sediment. By carefully loosening the finger, but not entirely removing it, a small amount of the sediment is then allowed to run slowly into the pipet. Slightly rotating the pipet will aid in accomplishing this, and at the same time will serve to loosen any structures which cling to the bottom of the tube. After wiping off the urine which adheres to the outside, a drop from the pipet is placed upon a clean slide. A hair is then placed in the drop, and a large cover-glass
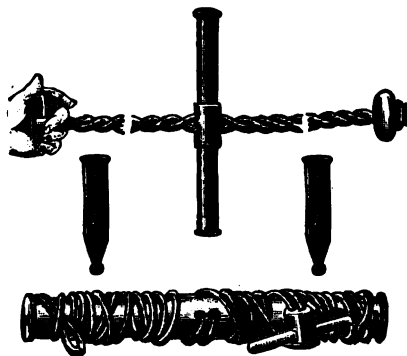
Fig. 35.—Wetherill's torfuge.

applied. Many workers use no cover. This offers a thicker layer and larger area of urine, the chance of finding scanty structures being proportionately increased. It has the disadvantage that any jarring of the room (as by persons walking about) sets the microscopic field into vibratory motion and makes it impossible to see anything clearly; and since it does not allow of the use of high-power objectives, one cannot examine details as one often wishes to do. A large cover-glass with a hair beneath it avoids these disadvantages, and gives enough urine to find any structures which are present in sufficient

number to have clinical significance, provided other
points in the technic have been right. It is best, how-
ever, to examine several drops; and, when the sediment
is abundant, drops from the upper and lower portions
should be examined separately.

In examining urinary sediments microscopically no
fault is so common, nor so fatal to good results, as im-
proper illumination (see Fig. 4), and none is so easily
corrected. The light should be central and very sub-
dued for ordinary work, but oblique illumination, ob-
tained by swinging the mirror a little out of the optical
axis, will be found helpful in identifying certain delicate
structures like hyaline casts. The 16 mm. objective
should be used as a finder, while the 4 mm. is reserved
for examining details. An experienced worker will rely
almost wholly upon the lower power.

It is well to emphasize that *the most common errors
which result in failure to find important structures, when
present, are lack of care in transferring the sediment to the
slide, too strong illumination, and too great magnification.*

In order to distinguish between similar structures it is
often necessary to watch the effect upon them of certain
reagents. This is especially true of the various unorgan-
ized sediments. They very frequently cannot be identi-
fied from their form alone. With the structures still in
focus, a drop of the reagent may be placed at one edge of
the cover-glass and drawn underneath it by the suction of
a piece of blotting-paper touched to the opposite edge;
or a small drop of the reagent and of the urine may be
placed close together upon a slide and a cover gently
lowered over them. As the two fluids mingle, the effect
upon various structures may be seen.

Urinary sediments may be studied under three heads:
A. Unorganized sediments. B. Organized sediments. C.
Extraneous structures.

A. UNORGANIZED SEDIMENTS

In general these have little diagnostic or prognostic
significance. Most of them are substances normally
present in solution, which have been precipitated either
because present in excessive amounts, or, more frequently,
because of some alteration in the urine (as in reaction,
concentration, etc.) which may be purely physiologic,
depending upon changes in diet or habits. Various
substances are always precipitated during decompo-
sition, which may take place either within or without

Fig. 36.—Unusual urinary crystals (drawn from various authors): 1, Calcium sul-
phate (colorless); 2, cholesterin (colorless); 3, hippuric acid (colorless); 4, hematoidin
(brown); 5, fatty acids (colorless); 6, indigo (blue); 7, sodium urate (yellowish).

the body. Unorganized sediments may be classified
according to the reaction of the urine in which they are
most likely to be found:

In acid urine: Uric acid, amorphous urates, sodium
urate, calcium oxalate, leucin and tyrosin, cystin, and
fat-globules. Uric acid, the urates, and calcium oxalate

are the common deposits of acid urines; the others are less frequent, and depend less upon the reaction of the urine.

In alkaline urine: Phosphates, calcium carbonate, and ammonium urate.

Other crystalline sediments (Fig. 36) which are rare and require no further mention are: Calcium sulphate, cholesterin, hippuric acid, hematoidin, fatty acids, and indigo.
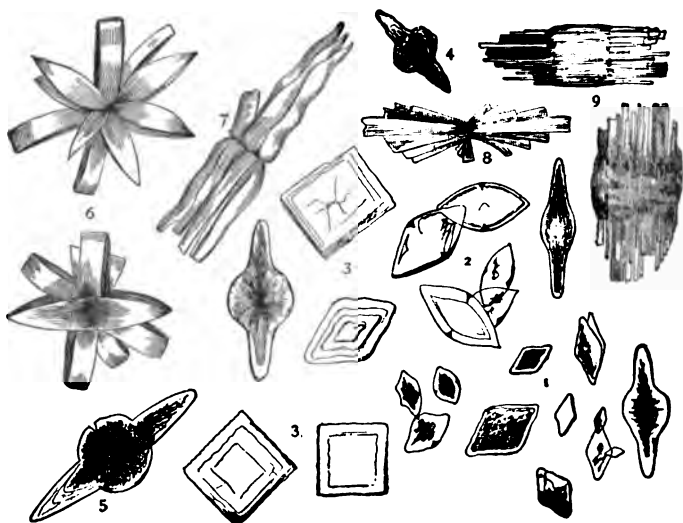
Fig. 37.—Forms of uric acid: 1, Rhombic plates; 2, whetstone forms; 3, 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosets; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine (Ogden).

1. In Acid Urine.—(1) Uric-acid Crystals.—These

crystals are the red grains—" gravel " or " red sand "—which are often seen adhering to the sides and bottom of a vessel containing urine.   Microscopically, they are yellow or reddish-brown crystals, which differ greatly in

PLATE III
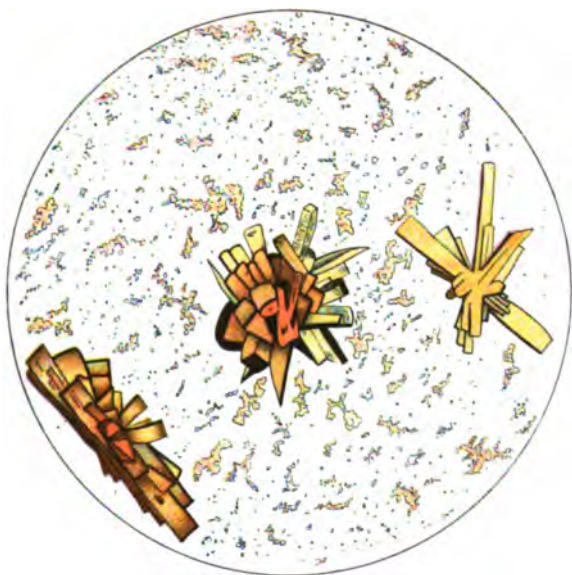
Uric-acid crystals with amorphous urates (after Peyer).
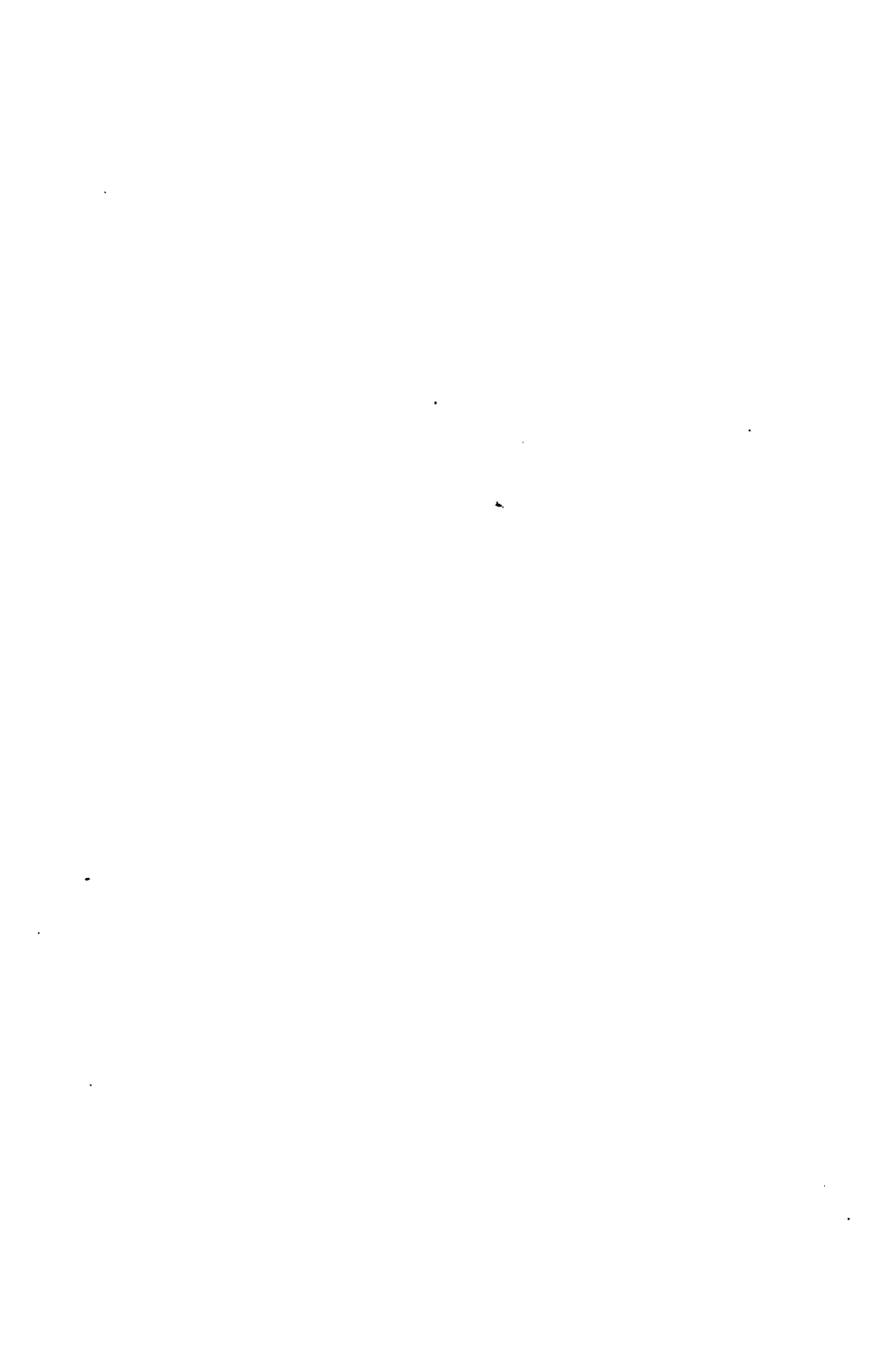
size and shape. The most characteristic forms (Plate III and Fig. 37) are " whetstones "; roset-like clusters of prisms and whetstones; and rhombic plates, which have usually a paler color than the other forms and are sometimes colorless. A very rare form is a colorless hexagonal plate resembling cystin. Recognition of the crystals depends less upon their shape than upon their color, the reaction of the urine, and the facts that they are soluble in caustic soda solution and insoluble in hydrochloric or acetic acid. When ammonia is added, they dissolve and crystals of ammonium urate appear.

A deposit of uric-acid crystals has no significance unless it occurs before or very soon after the urine is voided. Every urine, if kept acid, will in time deposit its uric acid. Factors which favor an early deposit are high acidity, diminished urinary pigments, and excessive excretion of uric acid. The chief clinical interest of the crystals lies in their tendency to form calculi, owing to the readiness with which they collect about any solid object. Their presence in the freshly voided urine in clusters of crystals suggests stone in the kidney or bladder, especially if blood is also present. (See Fig. 65.)

(2) **Amorphous Urates.**—These are chiefly urates of sodium and potassium which are thrown out of solution as a yellow or red " brick-dust " deposit. In pale urines this sediment is almost white. It disappears upon heating. A deposit of amorphous urates is very common in concentrated and strongly acid urines, especially in cold weather, and has no clinical significance. Under the microscope it appears as fine yellowish granules, often so abundant as to obscure all other structures (Plate III). In such cases the urine should be warmed

before examining. Amorphous urates are readily soluble in caustic soda solutions. When treated with hydrochloric or acetic acid, they slowly dissolve and rhombic crystals of uric acid appear.

Rarely, sodium urate occurs in crystalline form—slender prisms, arranged in fan- or sheaf-like structures (Fig. 36).

(3) **Calcium Oxalate.**—Characteristic of calcium oxalate are colorless, glistening, octahedral crystals, giving the appearance of small squares crossed by two intersect-
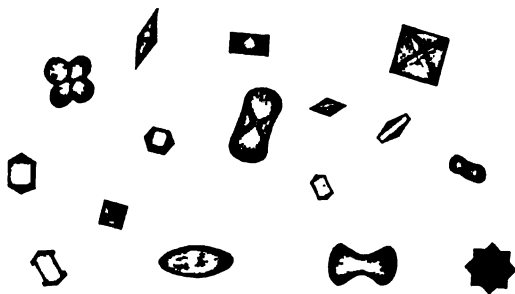
Fig. 38.—Various forms of calcium oxalate crystals (Ogden).

ing diagonal lines—the so-called " envelop crystals " (Fig. 51). They vary greatly in size, being sometimes so small as to seem mere points of light with medium-power objectives. Unusual forms, which, however, seldom occur except in conjunction with the octahedra, are colorless dumb-bells, spheres, and variations of the octahedra (Fig. 38). The spheres might be mistaken for globules of fat or red blood-corpuscles. Crystals of calcium oxalate are insoluble in acetic acid or caustic soda. They are dissolved by strong hydrochloric acid,

and recrystallize as octahedra upon addition of ammonia. They are sometimes encountered in alkaline urine.

The crystals are commonly found in the urine after ingestion of vegetables rich in oxalic acid, as tomatoes, spinach, asparagus, and rhubarb. They have no definite significance pathologically. They often appear in digestive disturbances, in neurasthenia, and when the oxidizing power of the system is diminished. When abundant, they are generally associated with a little mucus; and, in men, frequently with a few spermatozoa. Like uric acid, their chief clinical interest lies in their tendency to form calculi, and their presence in fresh urine, together with evidences of renal or cystic irritation, should be viewed with suspicion, particularly if they are clumped in small masses.

(4) **Leucin and Tyrosin.**—Crystals are deposited only when the substances are present in considerable amount. When present in smaller amount, they will usually be deposited if a little of the urine be slowly evaporated upon a slide. Addition of alcohol favors the deposit. They generally appear together, and are of comparatively rare occurrence, usually indicating severe fatty destruction of the liver, such as occurs in acute yellow atrophy and phosphorus-poisoning.

The crystals cannot be identified from their morphology alone, since other substances, notably calcium phosphate (Fig. 42) and ammonium urate, may take similar or identical forms.

Leucin crystals (Fig. 39) as they appear in the urine do not represent the pure substance. They are slightly yellow, oily-looking spheres, many of them with radial and concentric striations. Some may be merged to-

10

gether in clusters. They are not soluble in hydrochloric acid nor in ether.

Tyrosin crystallizes in very fine colorless needles, usually arranged in sheaves, with a marked constriction at the middle (Fig. 39). It is soluble in ammonia and hydrochloric acid, but not in acetic acid.

(5) **Cystin crystals** are colorless, highly refractive, rather thick, hexagonal plates with well-defined edges. They lie either singly or superimposed to form more or less irregular clusters (Fig. 40). Uric acid sometimes
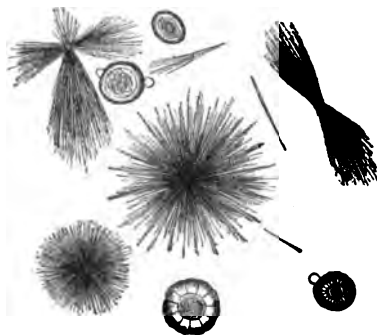
Fig. 39.—Leucin spheres and tyrosin needles (Stengel).

takes this form and must be excluded. Cystin is soluble in hydrochloric acid, insoluble in acetic; it is readily soluble in ammonia and recrystallizes upon addition of acetic acid.

Cystin is one of the amino-acids formed in decomposition of the protein molecule, and is present in traces in normal urine. Crystals are deposited only when the substance is present in excessive amount. Their presence is known as *cystinuria*. It is a rare condition due to an obscure abnormality of protein metabolism and usually

continues throughout life.   There are rarely any symp-
toms save those referable to renal or cystic calculus, to
which the condition strongly predisposes.

(6) **Fat-globules.**—Fat appears in the urine as highly
refractive globules of various sizes, frequently very small.
These globules are easily recognized from the fact that
they are stained black by osmic acid and orange or red
by Sudan III.   The stain may be applied upon the slide,
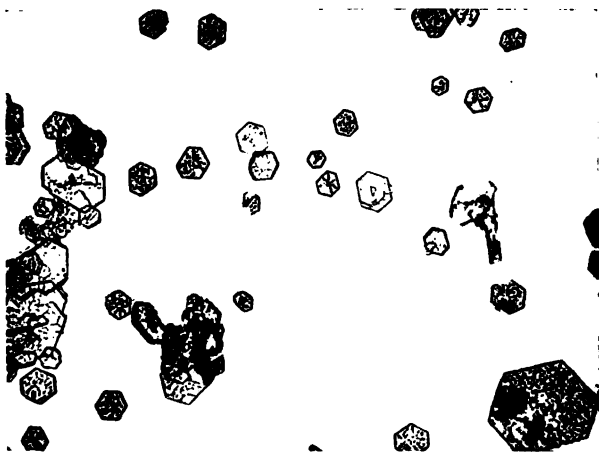
Fig. 40.—Cystin crystals from urine of patient with cystin calculus (X 200) (photograph by
the author).

as already described (p. 140).   Osmic acid should be
used in 1 per cent. aqueous solution; Sudan III in satu-
rated solution in 70 per cent. alcohol, to which one-half
volume of 10 per cent. formalin may advantageously
be added.

Fat in the urine is usually a contamination from un-
clean vessels, oiled catheters, etc.   A very small amount
may be present after ingestion of large quantities of cod-

liver oil or other fats. In fatty degeneration of the kidney, as in phosphorus-poisoning and chronic parenchymatous nephritis, fat-globules are commonly seen, both free in the urine and embedded in cells and tube-casts.

In *chyluria*, or admixture of chyle with the urine as a result of rupture of a lymph-vessel, minute droplets of fat are so numerous as to give the urine a milky appearance. The droplets are generally smaller than those of milk. The fluid is often blood-tinged. Chyluria occurs most frequently as a symptom of infection by filaria (p. 357), the embryos of which can usually be found in the milky urine.

2. In Alkaline Urine.—(1) **Phosphates.**—While most common in alkaline urine, phosphates are sometimes deposited in amphoteric or feebly acid urines. The usual forms are: (*a*) Ammoniomagnesium phosphate crystals; (*b*) acid calcium phosphate crystals; and (*c*) amorphous phosphates. All are readily soluble in acetic acid.

(*a*) *Ammoniomagnesium Phosphate Crystals.*—They are the common " triple phosphate " crystals, which are generally easily recognized (Figs. 41 and 66, and Plate IV). They are colorless, except when bile-stained. Their usual form is some modification of the prism, with oblique ends. Most typical are the well-known " coffinlid " and " hip-roof " forms. The long axis of the hip-roof crystal is often so shortened that it resembles the envelop crystal of calcium oxalate. It does not, however, have the same luster; this, and its solubility in acetic acid, will always prevent confusion.

When rapidly deposited, as by artificial precipitation, triple phosphate often takes feathery, star-, or leaf-like

forms. These gradually develop into the more common prisms. X-forms may be produced by partial solution of prisms.

(b) *Acid Calcium Phosphate Crystals.*—In feebly acid, amphoteric, or feebly alkaline urines acid calcium phosphate, wrongly called "neutral calcium phosphate," is not infrequently deposited in the form of colorless prisms arranged in stars and rosets (Fig. 42, 1). The individual prisms are usually slender, with one beveled,

Fig. 41.—Various forms of triple phosphate crystals (Ogden).

wedge-like end, but are sometimes needle-like. They may sometimes take forms resembling tyrosin (Fig. 42, 2), calcium sulphate, or hippuric acid, but are readily distinguished by their solubility in acetic acid.

Calcium phosphate often forms large, thin, irregular, usually granular, colorless plates, which are easily recognized (Fig. 42, 3).

(c) *Amorphous Phosphates.*—The earthy phosphates are thrown out of solution in most alkaline and many amphoteric urines as a white, amorphous sediment,

which may be mistaken for pus macroscopically.   Under
the microscope the sediment is seen to consist of numer-
ous colorless granules, distinguished from amorphous

Fig. 42.—Crystals of calcium phosphate: 1, Common form (copied from Rieder's
Atlas); 2, needles resembling tyrosin (drawn from nature); 3, large, irregular plates (from
nature).

urates by their color, their solubility in acetic acid, and
the reaction of the urine.

The various phosphatic deposits frequently occur
together.   They are sometimes due to excessive excre-

Fig. 43.—Indistinct crystalline sediment (dumb-bell crystals) of calcium carbonate.
Similar crystals are formed by calcium oxalate and calcium sulphate (after Funke).

tion of phosphoric acid, but usually merely indicate that
the urine has become, or is becoming, alkaline.   (See
Phosphates, p. 86.)

(2) **Calcium carbonate** may sometimes be mingled
with the phosphatic deposits, usually as amorphous

PLATE IV
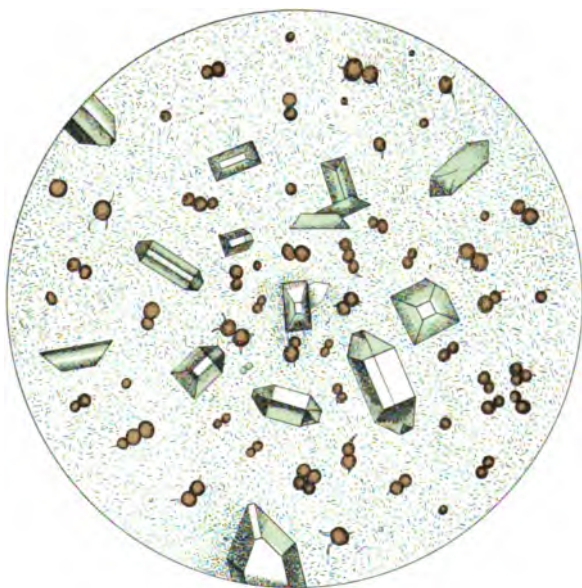
Sediment of alkaline fermentation (after Hofmann and Ultzmann).

granules, or, more rarely, as colorless spheres and dumb-bells (Fig. 43), which are soluble in acetic acid with gas-formation.

(3) **Ammonium Urate Crystals.**—This is the only urate deposited in alkaline urine. It forms opaque yellow crystals, usually in the form of spheres (Plate IV. and Fig. 66), which are often covered with fine or coarse
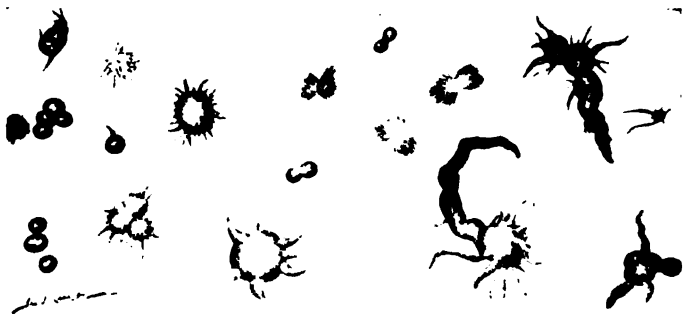
Fig. 44.—Crystals of ammonium urate (one-half of the forms copied from Rieder's Atlas, the others from nature).

spicules—" thorn-apple crystals." Sometimes dumb-bells, compact sheaves of fine needles, and irregular rhizome forms are seen (Fig. 44). Upon addition of acetic acid they dissolve, and rhombic plates of uric acid appear.

These crystals occur only when free ammonia is present. They are generally found along with the phosphates in decomposing urine and have no clinical significance.

B.   ORGANIZED SEDIMENTS

The principal organized structures in urinary sediments are: Tube-casts; epithelial cells; pus-corpuscles;

red blood-corpuscles; spermatozoa; bacteria, and animal parasites. They are much more important than the unorganized sediments just considered.

1. Tube-casts.—These interesting structures are albuminous casts of the uriniferous tubules. Their presence in the urine probably always indicates some pathologic change in the kidney, although this change may be very slight or transitory. Large numbers may be present in temporary irritations and congestions. *They do not in themselves, therefore, imply organic disease of the kidney.* They rarely occur in urine which does not contain, or has not recently contained, albumin.

While it is not possible to draw a sharp dividing-line between the different varieties, casts may be classified as follows:

(1) Hyaline casts.
      (*a*) Narrow.
      (*b*) Broad.
(2) Waxy casts.
(3) Fibrinous casts.
(4) Granular casts.
      (*a*) Finely granular.
      (*b*) Coarsely granular.
(5) Fatty casts.
(6) Casts containing organized structures.
      (*a*) Epithelial casts.
      (*b*) Blood-casts.
      (*c*) Pus-casts.
      (*d*) Bacterial casts.

As will be seen later, practically all varieties are modifications of the hyaline.

The significance of the different varieties is more readily understood if one considers their mode of formation. Albuminous material, the source and nature of which are not definitely known, but which are doubtless not the same in all cases, probably enters the lumen of a uriniferous tubule in a fluid or plastic state. The material has been variously thought to be an exudate from the blood, a pathologic secretion of the renal cells, and a product of epithelial degeneration. In the tubule it hardens into a cast which, when washed out by the urine, retains the shape of the tubule, and contains within its substance whatever structures and débris were lying free within the tubule or were loosley attached to its wall. If the tubule be small and have its usual lining of epithelium, the cast will be narrow; if it be large or entirely denuded of epithelium, the cast will be broad. *A cast, therefore, indicates the condition of the tubule in which it is formed, but does not necessarily indicate the condition of the kidney as a whole.*

The search for casts must be carefully made. The urine must be fresh, since hyaline casts soon dissolve when it becomes alkaline. It should be thoroughly centrifugalized. When the sediment is abundant, casts, being light structures, will be found near the top. In cystitis, where casts may be entirely hidden by the pus, the bladder should be irrigated to remove as much of the pus as possible and the next urine examined. In order to prevent solution of the casts the urine, if alkaline, must be rendered acid by previous administration of boric acid or other drugs. Heavy sediments of urates, blood, or vaginal cells may likewise obscure casts and other important structures. The last can be

avoided by catheterization. Urates can be dissolved by gently warming before centrifugalizing, care being taken not to heat enough to coagulate the albumin. The albumin shield of the centrifuge tube may also be heated. Blood can be destroyed by centrifugalizing, pouring off the supernatant urine, filling the tube with water, adding a few drops of dilute acetic acid, mixing well, and again centrifugalizing; this process being repeated until the blood is completely decolorized. Too much acetic acid will dissolve hyaline casts.

Their cylindric shape can be best seen by slightly moving the cover-glass while observing them, thus causing them to roll. This little manipulation should be practised until it can be done satisfactorily. It will prove useful in many examinations.

Various methods of staining casts so as to render them more conspicuous have been proposed. They offer no special advantage to one who understands how to use the substage mechanism of his microscope. The " negative-staining " method is as good as any. It consists simply in adding a little India-ink to the drop of urine on the slide. Casts, cells, etc., will stand out as colorless structures on a dark background.

(1) **Hyaline Casts.**—Typically, these are colorless, homogeneous, semitransparent, cylindric structures, with parallel sides and usually rounded ends. Not infrequently they are more opaque or show a few granules or an occasional oil-globule or cell, either adhering to them or contained within their substance. Generally they are straight or curved; less commonly, convoluted. Their length and breadth vary greatly: they are sometimes so long as to extend across several fields of a

medium-power objective, but are usually much shorter; in breadth, they vary from one to seven or eight times
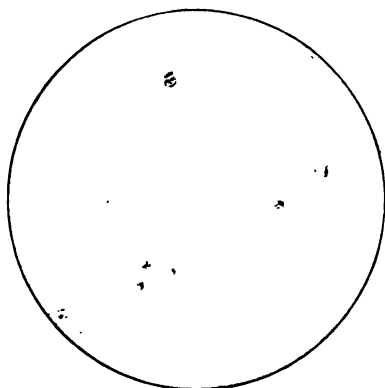
Fig. 45.—Hyaline casts showing fat-droplets and leukocytes (obj. one-sixth) (Boston).

the diameter of a red blood-corpuscle. (See Figs. 4, 45, 46, and 50.)

Fig. 46.—Various kinds of casts: *a*, Hyaline and finely granular cast; *b*, finely granular cast; *c*, coarsely granular cast; *d*, brown granular cast; *e*, granular cast with normal and abnormal blood adherent; *f*, granular cast with renal cells adherent; *g*, granular cast with fat and a fatty renal cell adherent (Ogden).

Hyaline casts are the least significant of all the casts, and occur in many slight and transitory conditions.

Small numbers are common following ether anesthesia, in fevers, after excessive exercise, and in congestions and irritations of the kidney. They are always present, and are usually stained yellow when the urine contains much bile. While they are found in all organic diseases of the kidney, they are most important in chronic interstitial nephritis. Here they are seldom abundant, but their constant presence is the most reliable urinary sign of the disease. Small areas of chronic interstitial change are probably responsible for the few hyaline casts so frequently found in the urine of elderly persons.

Fig. 47.—Waxy casts (upper part of figure). Fatty and fat-bearing casts (lower part of figure) (from Greene's "Medical Diagnosis").

Very broad hyaline casts commonly indicate complete desquamation of the tubular epithelium, such as occurs in the late stages of nephritis.

(2) **Waxy Casts.**—Like hyaline casts, these are homogeneous when typical, but frequently contain a few granules or an occasional cell. They are much more opaque than the hyaline variety, and are usually shorter and broader, with irregular, broken ends, and sometimes appear to be segmented. They are grayish or colorless, and have a dull, waxy look, as if cut from par-

affin (Figs. 47 and 64). They are sometimes composed of material which gives the amyloid reactions. Waxy casts are found in most advanced cases of nephritis, where they are an unfavorable sign. They are perhaps most frequently found in amyloid disease of the kidney, but are not distinctive of the disease, as is sometimes stated.

(3) **Fibrinous Casts.**—Casts which resemble waxy casts, but have a distinctly yellow color, as if cut from beeswax, are often seen in acute nephritis. They are

Fig. 48.—Granular and fatty casts and two compound granular cells (Stengel).

called fibrinous casts, but the name is inappropriate, as they are not composed of fibrin. They are often classed with waxy casts, but should be distinguished, as their significance is much less serious.

(4) **Granular Casts.**—These are merely hyaline casts in which numerous granules are embedded (Figs. 46, 48, and 50).

Finely granular casts contain many fine granules, are usually shorter, broader, and more opaque than the hyaline variety, and are more conspicuous. Their color is grayish or pale yellow.

Coarsely granular casts contain larger granules and are darker in color than the finely granular, being often dark brown owing to presence of altered blood-pigment. They are usually shorter and more irregular in outline, and more frequently have irregularly broken ends.

(5) **Fatty Casts.**—Small droplets of fat may at times be seen in any variety of cast. Those in which the drop-lets are numerous are called fatty casts (Figs. 47 and 48). The fat-globules are not difficult to recognize. Staining with osmic acid or Sudan (p. 147) will remove any doubt as to their nature.

The granules and fat-droplets seen in casts are prod-ucts of epithelial degeneration. Granular and fatty casts, therefore, always indicate partial or complete dis-integration of the renal epithelium. The finely granular variety is the least significant, and is found when the epithelium is only moderately affected. Coarsely granular, and especially fatty casts, if present in con-siderable numbers, indicate a serious parenchymatous nephritis.

(6) **Casts Containing Organized Structures.**—Cells and other structures are frequently seen adherent to a cast or embedded within it. (See Figs. 45 and 46). When numerous, they give name to the cast.

(*a*) *Epithelial casts* contain epithelial cells from the renal tubules. They always imply desquamation of epithelium, which rarely occurs except in parenchy-matous inflammations (Figs. 63 and 64). When the cells are well preserved they point to acute nephritis.

(*b*) *Blood-casts* contain red blood-corpuscles, usually much degenerated (Figs. 49 and 63). They always indicate hemorrhage into the tubules, which is most

common in acute nephritis or an acute exacerbation of a chronic nephritis.

(c) *Pus-casts* (see Fig. 65), composed almost wholly of pus-corpuscles, are uncommon, and point to a chronic suppurative process in the kidney.

(d) True *bacterial casts* are rare. They indicate a septic condition in the kidney. Bacteria may permeate a cast after the urine is voided.

Fig. 49.—Red blood-corpuscles and blood-casts (courtesy of Dr. A. Scott) (obj. one-sixth) (Boston)

Structures Likely to be Mistaken for Casts.—(1) **Mucous Threads.**—Mucus frequently appears in the form of long strands which slightly resemble hyaline casts (Fig. 50). They are, however, more ribbon-like, have less well-defined edges, and usually show faint longitudinal striations. Their ends taper to a point or are split or curled upon themselves, and are never evenly rounded, as is commonly the case with hyaline casts.

Such threads form a part of the nubecula of normal urine, and are especially abundant when calcium oxalate

crystals are present.   When there is an excess of mucus, as in irritations of the urinary tract, every field may be filled with an interlacing meshwork.

Mucous threads are microscopic and should not be confused with urethral shreds, which are macroscopic, and consist of a matrix of mucus in which many epithelial and pus-cells are embedded.

(2) **Cylindroids.**—-This name is sometimes given to the mucous threads just described, but is more properly

Fig. 50.—Hyaline and granular casts, mucous threads, and cylindroids.   There are also a few epithelial cells from the bladder (Wood).

applied to certain peculiar structures more nearly allied to casts.   They resemble hyaline casts in structure, but differ in being broader at one end and tapering to a slender tail, which is often twisted or curled upon itself (Fig. 50).   They frequently occur in the urine along with hyaline casts, especially in irritations of the kidney, and have no definite pathologic significance.

(3) **Masses of amorphous urates, or phosphates, or**

very small crystals (Fig. 51), which accidentally take a cylindric form, or shreds of mucus covered with granules, closely resemble granular casts. Application of gentle heat or appropriate chemicals will serve to differentiate them. When urine contains both mucus and granules, large numbers of these " pseudocasts," all lying in the same direction, can be produced by slightly moving the cover-glass from side to side. It is possible—as in urate infarcts of infants—for urates to be molded into cylindric bodies within the renal tubules.

Fig. 51.—Calcium oxalate crystals, showing a pseudocast of small crystals (Jakob).

(4) **Hairs** and **fibers** of wool, cotton, etc. These could be mistaken for casts only by beginners. One can easily become familiar with their appearance by suspending them in water and examining with the microscope (Fig. 61).

(5) **Hyphæ** of **molds** are not infrequently mistaken for hyaline casts. Their higher degree of refraction, their jointed or branching structure, and the accompanying spores will differentiate them (Fig. 62).

11

2. Epithelial Cells.—A few cells from various parts of the urinary tract occur in every urine. A marked increase indicates some pathologic condition at the site of their origin. It is sometimes, but by no means always, possible to locate their source from their form. Most cells are much altered from their original shape. Any epithelial cell may be so granular from degenerative changes that the nucleus is obscured. They are usually divided into three groups:
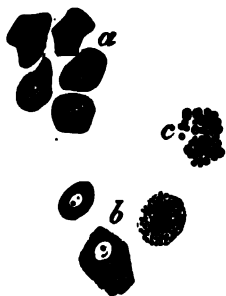
Fig. 52.—Renal epithelium from nephritic urine: *a*, Polyhedral epithelium in nephritis of scarlet fever; *b* and *c*, different grades of fatty degeneration in renal epithelium in chronic nephritis (× 400) (after Bizzozero).

(1) **Small, round** or **polyhedral cells** are about the size of pus-corpuscles, or a little larger, with a single round nucleus. Such cells may come from the deeper layers of any part of the urinary tract. They are uncommon in normal urine. When they are dark in color, very granular, and contain a comparatively large nucleus, they probably come from the renal tubules, but their origin in the kidney is not proved unless they are found embedded in casts. Renal cells are abundant in parenchymatous nephritis, especially the acute form. They are nearly always fatty—most markedly so in chronic parenchymatous nephritis, where their substance is sometimes wholly replaced by fat-droplets (" compound granule cells ") (see Figs. 48, 52, and 63).

(2) **Irregular cells** are considerably larger than the preceding. They are round, pear shaped, or spindle shaped, or may have tail-like processes, and are hence

named large round, pyriform, spindle, or caudate cells respectively. Each contains a round or oval distinct nucleus. Their usual source is the deeper layers of the urinary tract, especially of the bladder. Caudate forms come most commonly from the pelvis of the kidney (see Figs. 53, *b*, 54, 65, and 66).

(3) **Squamous** or **pavement cells** are large flat cells, each with a small, distinct, round or oval nucleus (Fig. 53, *a*). They are derived from the superficial layers of

Fig. 53.—Epithelial cells from urethra and bladder: *a*, Squamous cells from superficial layers; *b*, irregular cells from deeper layers (Jakob).

the ureters, bladder, urethra, or vagina, and when desquamation is active, appear in stratified masses. Squamous cells from the bladder are generally rounded, while those from the vagina are larger, thinner, and more angular. Great numbers of these vaginal cells, together with pus-corpuscles, may be present when leukorrhea exists.

3. Pus-corpuscles.—A very few leukocytes are present in normal urine. They are more abundant when

mucus is present. An excess of leukocytes, mainly of the polymorphonuclear variety, with albumin, constitutes *pyuria*—pus in the urine.
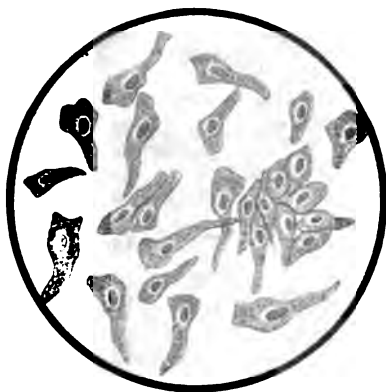
Fig. 54.—Caudate epithelial cells from pelvis of kidney (Jakob).

When at all abundant, pus forms a white sediment resembling amorphous phosphates macroscopically. Un-
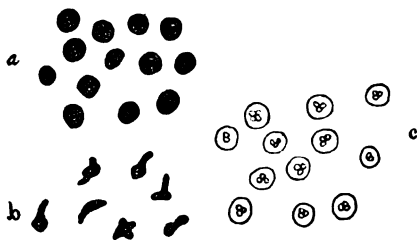
Fig. 55.—Pus-corpuscles: *a*, As ordinarily seen; *b*, ameboid corpuscles; *c*, showing the action of acetic acid (Ogden).

der the microscope the corpuscles appear as very granular cells, about twice the diameter of a red blood-corpuscle (Figs. 55 and 66). In freshly voided urine many

exhibit ameboid motion, assuming irregular outlines. Each contains one irregular nucleus or several small, rounded nuclei. The nuclei are obscured or entirely hidden by the granules, but may be brought clearly into view by running a little acetic acid under the cover-glass. This enables one to easily distinguish pus-cor-puscles from small round epithelial cells, which resemble them in size, but have a single, rather large, round nucleus. In decomposing urine pus is converted into a gelatinous mass which gives the urine a ropy consistence.

Pyuria indicates suppuration in some part of the urinary tract—urethritis, cystitis, pyelitis, etc.—or may be due to contamination from the vagina, in which case many vaginal epithelial cells will also be present. In general, the source of the pus can be determined only by the accompanying structures (epithelia, casts) or by the clinical signs.

A fairly accurate idea of the quantity of pus from day to day may be had by shaking the urine thoroughly and counting the number of corpuscles per cubic millimeter upon the Thoma-Zeiss blood-counting slide.

4. Red Blood-corpuscles.—Urine which contains blood is always albuminous. Very small amounts do not alter its macroscopic appearance. Larger amounts alter it considerably. Blood from the kidneys is generally intimately mixed with the urine and gives it a hazy reddish or brown color. When from the lower urinary tract, it is not so intimately mixed and settles more quickly to the bottom, the color is brighter, and small clots are often present.

Red blood-corpuscles are not usually difficult to recognize with the microscope. When very fresh, they have a

normal appearance, being yellowish discs of uniform size (normal blood). When they have been in the urine any considerable time, their hemoglobin may be dissolved out, and they then appear as faint colorless circles or " shadow cells " (abnormal blood), and are more difficult to see (Fig. 56; see also Figs. 49 and 63). They are apt to be swollen in dilute and crenated in concentrated urines. The microscopic findings may be corroborated by chemic tests for hemoglobin, although the microscope may show a few red corpuscles when the chemic tests are negative.

When not due to contamination from menstrual discharge, blood in the urine, or *hematuria*, is always patho-
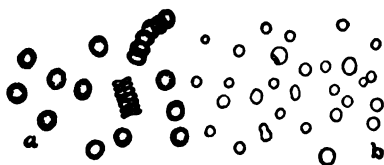
Fig. 56.—Blood-corpuscles: *a*, Normal; *b*, abnormal (Ogden).

logic. Blood comes from the *kidney tubules* in severe hyperemia, in acute nephritis and acute exacerbations of chronic nephritis, and in renal tuberculosis and malignant disease. An " idiopathic hematuria," probably of nervous origin, has been observed. The finding of blood-casts is the only certain means of diagnosing the kidney as its source. Blood comes from the *pelvis of the kidney* in renal calculus (Fig. 65), and is then usually intermittent, small in amount, and accompanied by a little pus and perhaps crystals of the substance forming the stone. Considerable hemorrhages from the *bladder* may occur in vesical calculus, tuberculosis, and new growths. Small amounts of blood generally accompany acute

cystitis. In Africa the presence of *Schistosomum hema-tobium* in the veins of the bladder is a common cause of hemorrhage (Egyptian hematuria).

5. Spermatozoa are generally present in the urine of men after nocturnal emissions, after epileptic convulsions, and in spermatorrhea. They may be found in the urine of both sexes following coitus. They are easily recognized from their characteristic structure (Fig. 57).

Fig. 57.—Spermatozoa in urine (Ogden).

The 4 mm. objective should be used, with subdued light and careful focusing.

6. Bacteria.—Normal urine is free from bacteria in the bladder, but becomes contaminated in passing through the urethra. Various non-pathogenic bacteria, notably *Micrococcus ureæ* (Fig. 58), are always present in decomposing urine. In suppurations of the urinary tract pus-producing organisms may be found. In many

infectious diseases the specific bacteria may be eliminated
in the urine without producing any local lesion.   Ty-
phoid bacilli have been known to persist for months
and even years after the attack.

Bacteria produce a cloudiness which will not clear
upon filtration.   They are easily seen with the 4 mm.
objective in the routine microscopic examination.
Ordinarily, no attempt is made to identify any but the
tubercle bacillus and the gonococcus.

Fig. 58.—Micrococcus ureæ (after von Jaksch).

Tubercle bacilli are nearly always present in the urine
when tuberculosis exists in any part of the urinary tract,
but are often difficult to find, especially when the urine
contains little or no pus.

Detection of Tubercle Bacilli in Urine.—The urine should
be obtained by catheter after careful cleansing of the parts.

(1) Centrifugalize thoroughly, after dissolving any sediment
of urates or phosphates by gentle heat or acetic acid.   Pour
off the supernatant fluid, add water, and centrifugalize again.
Addition of one or two volumes of alcohol will favor cen-
trifugalization by lowering the specific gravity.

(2) Make thin smears of the sediment, adding a little egg-
albumen if necessary to make the smear adhere to the glass;
dry, and fix in the usual way.

(3) Stain with carbol-fuchsin, steaming for at least three
minutes, or at room temperature for six to twelve hours.

PLATE V

Tubercle bacilli in urinary sediment; $\times$ 800 (Ogden).
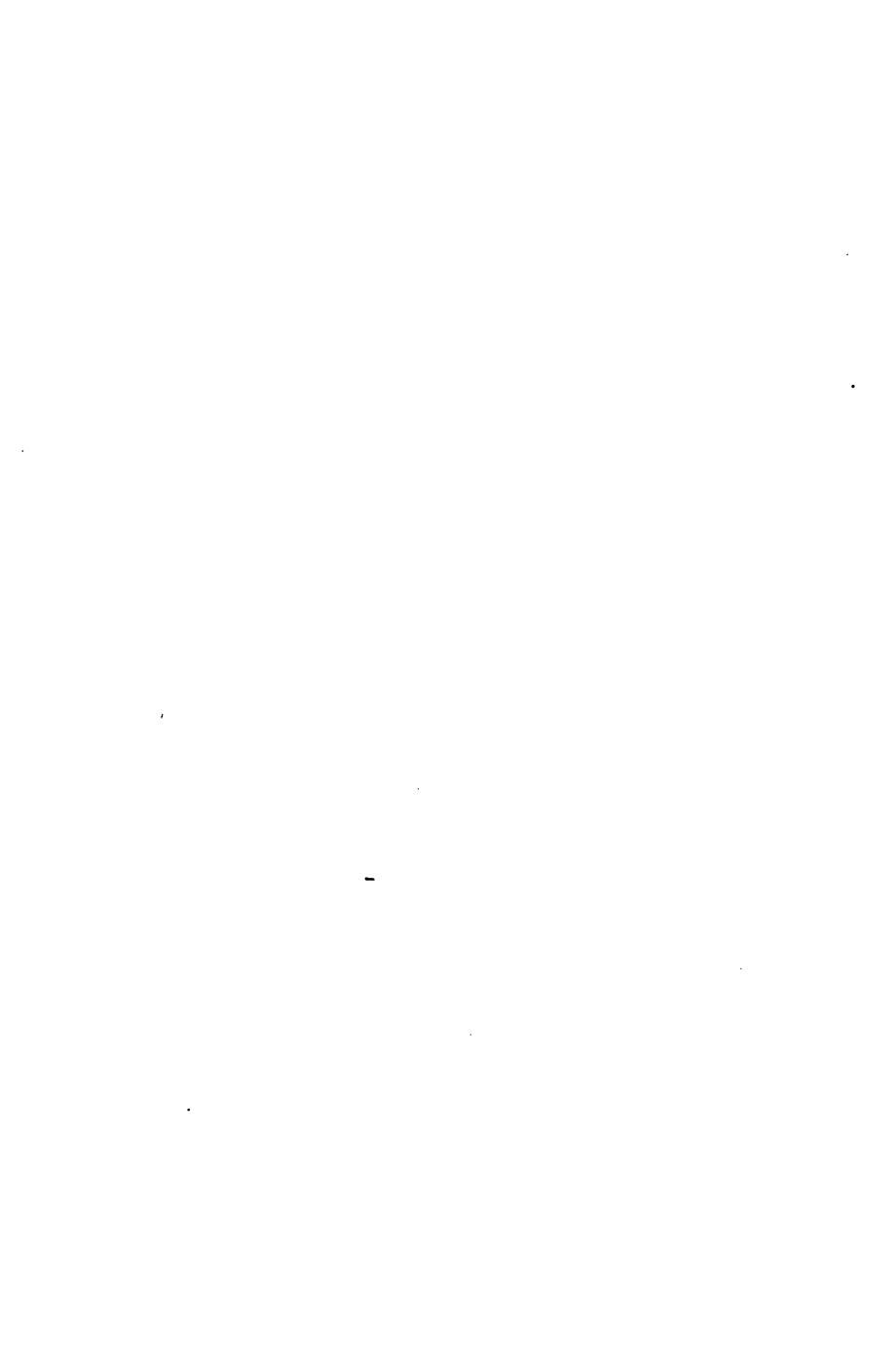
(4) Wash in water, and then in 20 per cent. nitric acid until only a faint pink color remains.

(5) Wash in water.

(6) Soak in alcohol fifteen minutes or longer. This decolorizes the smegma bacillus (p. 53), which is often present in the urine, and might easily be mistaken for the tubercle bacillus. It is unlikely, however, to be present in catheterized specimens. It is always safest to soak the smear in alcohol for several hours or over night, since some strains of the smegma bacillus are very resistant.

(7) Wash in water.

(8) Apply Löffler's methylene-blue solution one-half minute.

(9) Rinse in water, dry between filter-papers, and examine with the one-twelfth objective.

When the bacilli are scarce, the following method may be tried. It is applicable also to other fluids. If the fluid is not albuminous, add a little egg-albumen. Coagulate the albumen by gentle heat and centrifugalize. The bacilli will be carried down with the albumen. The sediment is then treated by the antiformin method (p. 52).

A careful search of many smears may be necessary to find the bacilli. They usually lie in clusters (see Plate V). Failure to find them in suspicious cases should be followed by inoculation of guinea-pigs; this is the court of last appeal, and must also be sometimes resorted to in order to exclude the smegma bacillus.

In gonorrhea **gonococci** are sometimes found in the sediment, but more commonly in the " gonorrheal threads," or " floaters." In themselves, these threads are by no means diagnostic of gonorrhea. Detection of the gonococcus is described later (p. 369).

7. Animal parasites are rare in the urine. Hooklets and scolices of *Tænia echinococcus* (Fig. 59) and em-

bryos of filariæ have been met. In Africa the ova, and even adults, of *Schistosomum hæmatobium* are common,

Fig. 59.—1, Scolex of tænia echinococcus, showing crown of hooklets; 2, scolex and detached hooklets (obj. one-sixth) (Boston).

accompanying " Egyptian hematuria." *Trichomonas vaginalis* is a not uncommon contamination. This and

Fig. 60.—Embryo of "vinegar eel" in urine, from contamination; length, 340 μ; width, 15 μ. An epithelial cell from bladder and three leukocytes are also shown (studied with Dr. J. A. Wilder).

other protozoa may be mistaken for spermatozoa by the inexperienced.

A worm which is especially interesting is *Anguillula aceti*, the "vinegar eel." This is generally present in the sediment of table vinegar, and may reach the urine through use of vinegar in vaginal douches, or through contamination of the bottle in which the urine is contained. It has been mistaken for *Strongyloides intestinalis* and for the filaria embryo. It closley resembles the former in both adult and embryo stages. The young embryos have about the same length as filaria embryos, but are nearly twice as broad and the intestinal canal is easily seen (compare Figs. 60 and 134). For fuller descriptions of these parasites the reader is referred to Chapter VI.

C. EXTRANEOUS STRUCTURES

The laboratory worker must familiarize himself with the microscopic appearance of the more common of the numerous structures which may be present from accidental contamination (Fig. 61).

Yeast-cells are smooth, colorless, highly refractive, spheric or ovoid cells. They sometimes reach the size of a leukocyte, but are generally smaller (see Fig. 106, *l*). They might be mistaken by the inexperienced for red blood-corpuscles, fat-droplets, or the spheric crystals of calcium oxalate, but are distinguished by the facts that they are not of uniform size; that they tend to adhere in short chains; that small buds may often be seen adhering to the larger cells; and that they do not give the hemoglobin test, are not stained by osmic acid or Sudan, but are colored brown by Lugol's solution, and are insoluble in acids and alkalis. Yeast-cells multiply rapidly

in diabetic urine, and may reach the bladder and multiply there.

Mold fungi (Fig. 62) are characterized by refractive, jointed, or branched rods (hyphæ), often arranged in a network, and by highly refractive, spheric or ovoid spores.
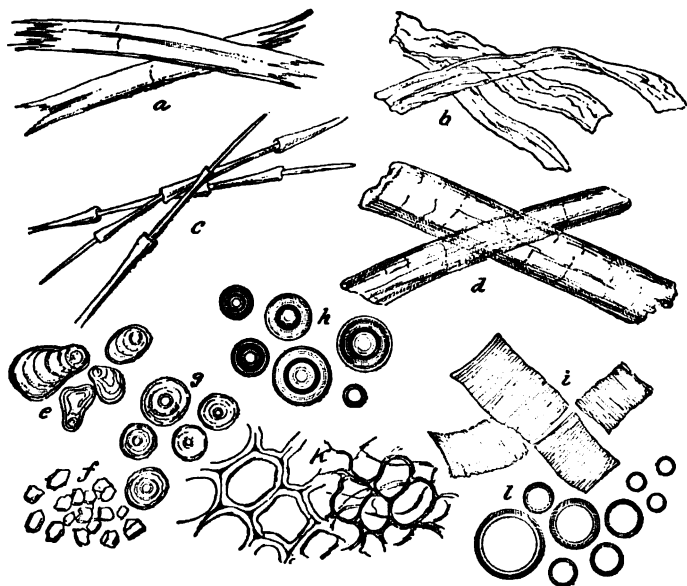
Fig. 61.—Extraneous matters found in urine: *a*, Flax-fibers; *b*, cotton-fibers; *c*, feathers; *d*, hairs; *e*, potato-starch; *f*, rice-starch granules; *g*, wheat-starch; *h*, air-bubbles; *i*, muscular tissue; *k*, vegetable tissue; *l*, oil-globules.

They are common in urine which has stood exposed to the air.

Fibers of wool, cotton, linen, or silk, derived from towels, the clothing of the patient, or the dust in the air, are present in almost every urine. *Fat-droplets* are most frequently derived from unclean bottles or oiled cathe-

ters. *Starch-granules* may reach the urine from towels, the clothing, or dusting-powders. They are recognized by their concentric striations and their blue color with iodin solution. *Lycopodium granules* (Fig. 5) may also reach the urine from dusting-powders. They might be mistaken for the ova of parasites. *Bubbles of air* are often confusing to beginners, but are easily recognized

Fig. 62.—Aspergillus from urine (Boston).

after once being seen. *Scratches* and *flaws* in the glass of slide or cover are likewise a common source of confusion to beginners.

IV. THE URINE IN DISEASE

In this section the characteristics of the urine in those diseases which produce distinctive urinary changes will be briefly reviewed.

1. **Renal Hyperemia.**—*Active hyperemia* is usually an early stage of acute nephritis, but may occur independently as a result of temporary irritation. The urine is generally decreased in quantity, highly colored, and strongly acid. Albumin is always present—usually in traces only, but sometimes in considerable amount for a

day or two. The sediment contains a few hyaline and finely granular casts and an occasional red blood-cell.

Fig. 63.—Sediment from acute hemorrhagic nephritis: Red blood-corpuscles; leukocytes; renal cells not fattily degenerated; epithelial and blood-casts (Jakob).

Fig. 64.—Sediment from chronic parenchymatous nephritis: Hyaline (with cells attached), waxy, brown granular, fatty, and epithelial casts; fattily degenerated renal cells, and a few white and red blood-corpuscles (Jakob).

In very severe hyperemia the urine approaches that of acute nephritis.

Passive hyperemia occurs most commonly in diseases of

the heart and liver and in pregnancy. The quantity of urine is somewhat low and the color high, except in pregnancy. Albumin is present in small amount only. The sediment contains a very few hyaline or finely granular casts. In pregnancy the amount of albumin should be carefully watched, as any considerable quantity, and especially a rapid increase, strongly suggests approaching eclampsia.

2. **Nephritis.**—The various degenerative and inflammatory conditions grouped under the name of nephritis have certain features in common. The urine in all cases contains albumin and tube-casts, and in all well-marked cases shows a decrease of normal solids, especially of urea and the chlorids. In chronic nephritis, especially of the interstitial type, there may be remissions during which the urine is practically normal. The characteristics of the different forms are well shown in the table on page 176, modified from Hill.

3. **Renal Tuberculosis.**—The urine is pale, usually cloudy. The quantity may not be affected, but is apt to be increased. In early cases the reaction is faintly acid and there are traces of albumin and a few renal cells. In advanced cases the urine is alkaline, has an offensive odor, and is irritating to the bladder. Albumin in varying amounts is always present. Pus is nearly always present, though frequently not abundant. It is generally intimately mixed with the urine, and does not settle so quickly as the pus of cystitis. Casts, though present, are rarely abundant, and are obscured by the pus. Small amounts of blood are common. Tubercle bacilli are nearly always present, although animal inoculation may be necessary to detect them.

THE URINE IN NEPHRITIS

	PHYSICAL.	CHEMIC.	MICROSCOPIC.
Acute nephritis.	Quantity diminished, often very greatly. Color dark; may be red or smoky. Specific gravity, 1.020 to 1.030.	Urea and chlorids low. Much albumin: up to 1.5 per cent. Reaction acid.	Sediment abundant, red or brown. Many casts, chiefly granular, blood and epithelial varieties. Red blood-cells abundant. Numerous renal epithelial cells and leukocytes.
Chronic parenchymatous nephritis. (Large white kidney.)	Quantity usually diminished. Color variable, often pale and hazy. Specific gravity, 1.010 to 1.020.	Urea and chlorids low. Largest amounts of albumin: up to 3 per cent. Reaction acid.	Sediment rather abundant. Many casts of all varieties: fatty casts and casts of degenerated epithelium most characteristic. Blood present in traces: abundant only in acute exacerbations. Numerous fatily degenerated renal epithelial cells, often free globules of fat, and a few leukocytes.
Chronic interstitial nephritis. (Contracted kidney.)	Quantity markedly increased, especially at night. Color pale, clear. Specific gravity, 1.005 to 1.015.	Urea and chlorids low in well-marked cases. Albumin present in traces (often overlooked), increasing in late stages. Reaction acid.	Sediment very slight. Few narrow hyaline and finely granular casts. No blood except in acute exacerbations. Very few renal cells. Uric acid and calcium-oxalate crystals common.
Amyloid degeneration of kidney.	Quantity moderately increased. Color pale, clear. Specific gravity, 1.012 to 1.018.	Slight decrease of urea and chlorids. Variable amounts of albumin and globulin.	Sediment slight. Moderate number of hyaline, finely granular, and sometimes waxy casts.

4. **Renal Calculus.**—The urine is usually somewhat concentrated, with high color and strongly acid reaction. Small amounts of albumin and a few casts may be present as a result of kidney irritation. Blood is frequently present, especially in the daytime and after severe exercise. Crystals of the substance composing the calculus—uric acid, calcium oxalate, cystin—may often be found. The presence of a calculus generally produces

Fig. 65.—Sediment from calculous pyelitis: Numerous pus-corpuscles, red blood-corpuscles, and caudate and irregular epithelial cells; a combination of hyaline and pus-casts, and a few uric-acid crystals (Jakob).

pyelitis, and variable amounts of pus then appear, the urine remaining acid in reaction.

5. **Pyelitis.**—In pyelitis the urine is slightly acid, and contains a small or moderate amount of pus, together with many spindle and caudate epithelial cells. Pus-casts may appear if the process extends up into the kidney tubules (see Fig. 65). Albumin is always present, and its amount, in proportion to the amount of pus, is decidedly greater than is found in cystitis. This fact is

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of much value in differential diagnosis.   Even when pus
is scanty, albumin is rarely under 0.15 per cent., which
is the maximum amount found in cystitis with abundant
pus.

6. **Cystitis.**—In *acute* and *subacute* cases the urine is
acid and contains a variable amount of pus, with many
epithelial cells from the bladder—chiefly large round,
pyriform, and rounded squamous cells.   Red blood-
corpuscles are often numerous.

Fig. 66.—Sediment from cystitis (chronic): Numerous pus-corpuscles, epithelial cells
from the bladder, and bacteria; a few red blood-corpuscles and triple phosphate and
ammonium urate crystals (Jakob).

In *chronic* cases the urine is generally alkaline.   It is
pale and cloudy from the presence of pus, which is abun-
dant and settles readily into a viscid sediment.   The
sediment usually contains abundant amorphous phos-
phates and crystals of triple phosphate and ammonium
urate.   Vesical epithelium is common.   Numerous bac-
teria are always present (see Fig. 66).

7. **Vesical Calculus, Tumors, and Tuberculosis.**—
These conditions produce a chronic cystitis, with its

characteristic urine.   Blood, however, is more frequently present and more abundant than in ordinary cystitis. With neoplasms, especially, considerable hemorrhages are apt to occur.   Particles of the tumor are sometimes passed with the urine.   No diagnosis can be made from the presence of isolated tumor cells.   In tuberculosis tubercle bacilli can generally be detected.

8. **Diabetes Insipidus.**—Characteristic of this disease is the continued excretion of very large quantities of pale, watery urine, containing neither albumin nor sugar. The specific gravity varies between 1.001 and 1.005. The daily output of solids, especially urea, is increased.

9. **Diabetes Mellitus.**—The quantity of urine is very large.   The color is generally pale, while the specific gravity is nearly always high—1.030 to 1.050, very rarely below 1.020.   The presence of glucose is the essential feature of the disease.   The amount of glucose is often very great, sometimes exceeding 8 per cent., while the total elimination may exceed 500 gm. in twenty-four hours.   It may be absent temporarily.   Acetone is generally present in advanced cases.   Diacetic and oxybutyric acids may be present, and usually warrant an unfavorable prognosis.   Accompanying the acidosis there is a corresponding increase in amount of ammonia.

CHAPTER III

THE BLOOD

Preliminary Considerations.—The blood consists of a fluid of complicated and variable composition, the plasma, in which are suspended great numbers of microscopic structures: viz., red corpuscles, white corpuscles, blood-platelets, and blood-dust.

Red corpuscles, or *erythrocytes*, appear as biconcave discs, red when viewed by reflected light or in thick layer, and straw colored when viewed by transmitted light or in thin layer. They give the blood its red color. They are cells which have been highly differentiated for the purpose of carrying oxygen from the lungs to the tissues. This is accomplished by means of an iron-bearing protein, hemoglobin, which they contain. In .the lungs hemoglobin forms a loose combination with oxgyen, which it readily gives up when it reaches the tissues. Normal erythrocytes do not contain nuclei. They are formed from preëxisting nucleated cells in .the bone-marrow.

White corpuscles, or *lcukocytes*, are less highly differentiated cells. There are several varieties. They all contain nuclei, and most of them contain granules which vary in size and staining properties. They are formed chiefly in the bone-marrow and lymphoid tissues.

Blood-platelets, or *blood-plaques*, are colorless or slightly bluish, spheric or ovoid bodies, about one-third or one-

half the diameter of an erythrocyte. Their structure, nature, and origin have not been definitely determined.

The *blood-dust of Müller* consists of fine granules which have vibratory motion. Little is known of them. It has been suggested that they are granules from disintegrated leukocytes.

The *total amount* of blood is usually given as one-thirteenth of the body weight, but more recent investigations indicate that it averages about one-twentieth.

The *reaction* is alkaline to litmus.

The *color* is due to the presence of hemoglobin in the red corpuscles, the difference between the bright red of arterial blood and the purplish red of venous blood depending upon the relative proportions of oxygen and carbon dioxid. The depth of color depends upon the amount of hemoglobin. In very severe anemias the blood may be so pale as to be designated as " watery." The formation of carbon-monoxid-hemoglobin in coalgas poisoning gives the blood a bright cherry-red color; while formation of methemoglobin in poisoning with potassium chlorate and certain other substances gives a chocolate color.

Coagulation consists essentially in the transformation of fibrinogen, one of the proteins of the blood, into fibrin by means of a ferment derived from disintegration of the leukocytes. The presence of calcium salts is necessary to the process. The resulting coagulum is made up of a meshwork of fibrin fibrils with entangled corpuscles and plaques. The clear, straw-colored fluid which is left after separation of the coagulum is called *blood-serum*. Normally, coagulation takes place in two to eight minutes after the blood leaves the vessels. It is frequently

desirable to determine the coagulation time. The simplest method is to place a drop of blood upon a perfectly clean slide, and to draw a needle through it at half-minute intervals. When the clot is dragged along by the needle, coagulation has taken place. This method is probably sufficient for ordinary clinical work. For very accurate results the method of Russell and Brodie, as modified by Boggs, is recommended. The instrument is shown in Fig. 67. A drop of blood is placed upon the cone, which is then quickly inverted in the moist chamber.
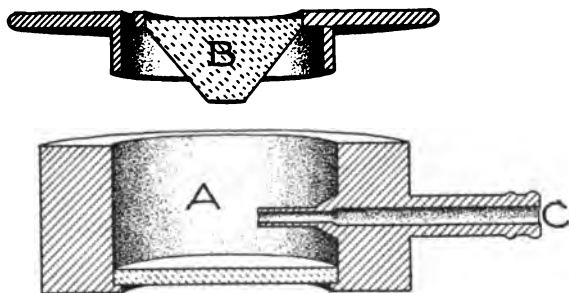
Fig. 67.—Boggs' coagulation instrument: A, moist chamber; B, glass cone; C, tube through which air is blown.

By means of a rubber bulb puffs of air are blown against the blood at intervals, while the motion of the corpuscles is watched with a low-power objective. Coagulation is complete when the red cells move only en masse and spring back to their original position when the current ceases. Coagulation is notably delayed in hemophilia and icterus and after administration of citric acid. It is hastened by administration of calcium salts.

For certain purposes, especially in bacteriologic and opsonic work, it is desirable to prevent coagulation

of the blood that is withdrawn. This may be accomplished by receiving it directly into a solution of 1 per cent. sodium citrate (or ammonium oxalate) and 0.85 per cent. sodium chlorid. This precipitates the calcium salts which are necessary to coagulation.

For most clinical examinations only one drop of blood is required. This may be obtained from the lobe of the ear, the palmar surface of the tip of the finger, or, in the case of infants, the plantar surface of the great toe. In general, the finger will be found most convenient. With nervous children the lobe of the ear is preferable, as it prevents their seeing what is being done. An edematous or congested part should be avoided. The site

Fig. 68.—Daland's blood-lancet.

should be well rubbed with alcohol to remove dirt and epithelial débris and to increase the amount of blood in the part. After allowing sufficient time for the circulation to equalize, the skin is punctured with a blood lancet (of which there are several patterns upon the market) or some substitute, as a Hagedorn needle, aspirating needle, trocar, or a pen with one of its nibs broken off. Nothing is more unsatisfactory than an ordinary sewing-needle. The lancet should be cleaned with alcohol before and after using, but need not be sterilized. It must be very sharp. If the puncture be made with a *firm*, *quick*, *rebounding stroke*, it is practically painless. The first drop of blood which appears should

be wiped away, and the second used for examination. The blood should not be pressed out, since this dilutes it with serum from the tissues; but moderate pressure some distance above the puncture is allowable.

When a larger amount of blood is required, it may be obtained with a sterile hypodermic syringe from one of the veins at the elbow, as described on p. 245.

Clinical study of the blood may be discussed under the following heads: I. Hemoglobin. II. Enumeration of erythrocytes. III. Color index. IV. Volume index. V. Enumeration of leukocytes. VI. Enumeration of plaques. VII. Study of stained blood. VIII. Blood parasites. IX. Serum reactions. X. Tests for recognition of blood. XI. Special blood pathology.

I. HEMOGLOBIN

Hemoglobin is an iron-bearing protein. It is found only within the red corpuscles, and constitutes about 90 per cent. of their weight. The actual amount of hemoglobin is never estimated clinically: it is the relation which the amount present bears to the normal which is determined. Thus the expression, " 50 per cent. hemoglobin," when used clinically, means that the blood contains 50 per cent. of the normal. Theoretically, the normal would be 100 per cent., but with the methods of estimation in general use the blood of healthy persons ranges from 85 to 105 per cent.; these figures may, therefore, be taken as normal.

Increase of hemoglobin, or *hyperchromemia*, is uncommon, and is probably more apparent than real. It accompanies an increase in number of erythrocytes, and may be noted in change of residence from a lower to a

higher altitude; in poorly compensated heart disease with cyanosis; in concentration of the blood from any cause, as the severe diarrhea of cholera, and in " idiopathic polycythemia."

Decrease of hemoglobin, or *oligochromemia*, is very common and important. It is the most striking feature of the secondary anemias (p. 277). Here the hemoglobin loss may be slight or very great. In mild cases a slight decrease of hemoglobin is the only blood change noted. In very severe cases, especially in repeated hemorrhages, malignant disease, and infection by the hookworm and *Dibothriocephalus latus*, hemoglobin may fall to 15 per cent. Hemoglobin is always diminished, and usually very greatly, in chlorosis, pernicious anemia, and leukemia.

Estimation of hemoglobin is less tedious and usually more helpful than a red corpuscle count. It offers the simplest and most certain means of detecting the existence and degree of anemia, and of judging the effect of treatment in anemic conditions. Pallor, observed clinically, does not always denote anemia.

There are many methods, but none is entirely satisfactory. Those which are most widely used are here described.

(1) **Von Fleischl Method.**—The apparatus consists of a stand somewhat like the base and stage of a microscope (Fig. 69). Under the stage is a movable bar of colored glass, shading from pale pink at one end to deep red at the other. The frame in which this bar is held is marked with a scale of hemoglobin percentages corresponding to the different shades of red. By means of a rack and pinion, the color-bar can be moved from end to end beneath a round opening in the center

of the stage. A small metal cylinder, which has a glass bottom and which is divided vertically into two equal compartments, can be placed over the opening in the stage so that one of its compartments lies directly over the color-bar. Accompanying the instrument are a number of short capillary tubes in metal handles.

Having punctured the finger-tip or lobe of the ear, as already described, wipe off the first drop of blood, and from the
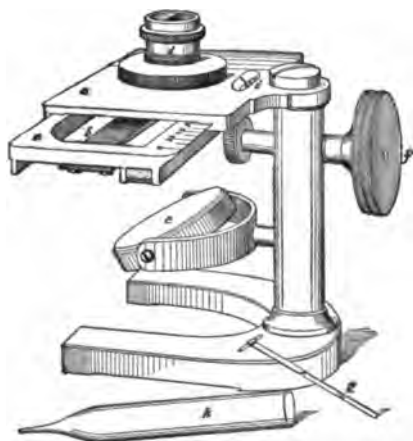
Fig. 69.—Von Fleischl's hemoglobinometer: *a*, Stand; *b*, narrow wedge-shaped piece of colored glass fitted into a frame (*c*), which passes under the chamber; *d*, hollow metal cylinder, divided into two compartments, which holds the blood and water; *e*, plaster-of-Paris plate from which the light is reflected through the chamber; *f*, screw by which the frame containing the graduated colored glass is moved; *g*, capillary tube to collect the blood; *h*, pipet for adding the water; *i*, opening through which may be seen the scale indicating percentage of hemoglobin.

second fill one of the capillary tubes. Hold the tube horizontally, and touch its tip to the drop of blood, which will readily flow into it if it be clean and dry. Avoid getting any blood upon its outer surface. With a medicine-dropper, rinse the blood from the tube into one of the compartments of the cylinder, using distilled water, and mix well. Fill both com-

partments level full with distilled water, and place the cylinder over the opening in the stage, so that the compartment which contains only water lies directly over the bar of colored glass. If there are any clots in the hemoglobin compartment, clean the instrument and begin again.

In a dark room, with the light from a candle reflected up through the cylinder, move the color-bar along with a jerking motion until both compartments have the same depth of color. The number upon the scale corresponding to the portion of the color-bar which is now under the cylinder gives the percentage of hemoglobin. While comparing the two colors, place the instrument so that they will fall upon the right and left halves of the retina, rather than upon the upper and lower halves; and protect the eye from the light with a cylinder of paper or pasteboard. After use, clean the metal cylinder with water, and wash the capillary tube with water, alcohol, and ether, successively. Results with this instrument are accurate to within about 5 per cent.

A recent modification of the von Fleischl apparatus by Miescher gives an error which need not exceed 1 per cent. It is, however, better adapted to laboratory use than to the needs of the practitioner.

(2) The **Sahli hemoglobinometer** (Fig. 70) is an improved form of the well-known Gowers instrument. It consists of a hermetically sealed comparison tube containing a 1 per cent. solution of acid hematin, a graduated test-tube of the same diameter, and a pipet of 20-c.mm. capacity. The two tubes are held in a black frame with a white ground-glass back.

Place a few drops of decinormal hydrochloric acid solution in the graduated tube. Obtain a drop of blood and draw it into the pipet to the 20 c.mm. mark. Wipe off the tip of the pipet, blow its contents into the hydrochloric acid solution in the tube, and rinse well. In a few minutes the hemoglobin is changed to acid hematin. Place the two tubes in the compartments of the frame, and dilute the fluid with water drop

by drop, mixing after each addition, until it has exactly the same color as the comparison tube. The graduation corresponding to the surface of the fluid then indicates the percentage of hemoglobin. Decinormal hydrochloric acid solution may be prepared with sufficient accuracy for this purpose

Fig. 70.—Sahli's hemoglobinometer.

by adding 15 c.c. of the concentrated acid to 985 c.c. distilled water. A little chloroform should be added as a preservative.

This method is very satisfactory in practice, and is accurate to within 5 per cent. The comparison tube is said to keep its color indefinitely, but, unfortunately, not all the instruments upon the market are well standardized.

(3) **Dare's hemoglobinometer** (Fig. 71) differs from the others in using undiluted blood. The blood is allowed to flow by capillarity into the slit between two small plates of glass. It is then placed in the instrument and compared with different portions of a circular disc of colored glass. The reading must be made quickly, before clotting takes place. This instrument is easy to use, and is one of the most accurate.
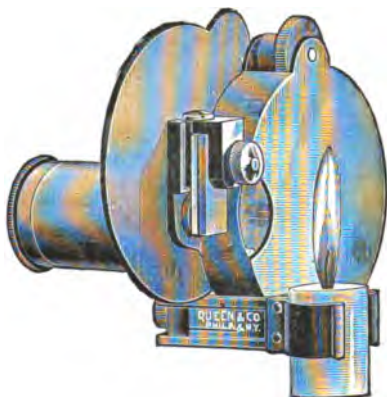
Fig. 71.—Dare's hemoglobinometer.

(4) **Hammerschlag Method.**—This is an indirect method which depends upon the fact that the percentage of hemoglobin varies directly with the specific gravity of the blood. It yields fairly accurate results except in leukemia, where the large number of leukocytes disturbs the relation, and in dropsical conditions.

Mix chloroform and benzol in a urinometer tube, so that the specific gravity of the mixture is near the probable specific gravity of the blood. Add a drop of blood by means of a pipet of small caliber. A pipet like that shown in Fig. 161, A will be found satisfactory. If the drop floats near the surface, add a little benzol; if it sinks to the bottom, add a little chloroform. When it remains stationary near the middle, the mix-

ture has the same specific gravity as the blood. Take the specific gravity with a urinometer, and obtain the corresponding percentage of hemoglobin from the following table:

SPECIFIC GRAVITY.	HEMOGLOBIN PER CENT.	SPECIFIC GRAVITY.	HEMOGLOBIN PER CENT.
1.033–1.035	25–30	1.048–1.050	55–65
1.035–1.038	30–35	1.050–1.053	65–70
1.038–1.040	35–40	1.053–1.055	70–75
1.040–1.045	40–45	1.055–1.057	75–85
1.045–1.048	45–55	1.057–1.060	85–95

For accurate results with this method, care and patience are demanded. The following precautions must be observed:
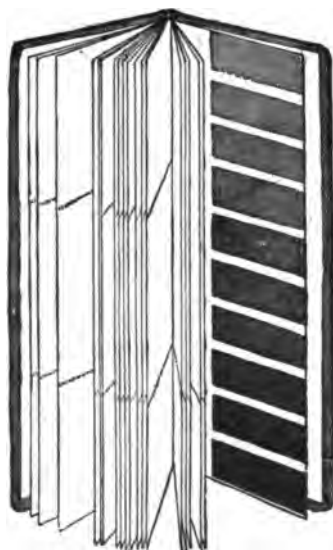
Fig. 72.—Tallquist's hemoglobin scale.

(a) The two fluids must be well mixed after each addition of chloroform or benzol. Close the tube with the thumb and invert several times. Should this cause the drop of blood to

break up into very small ones, adjust the specific gravity as accurately as possible with these, and test it with a fresh drop.

(*b*) The drop of blood must not be too large; it must not contain an air-bubble, it must not adhere to the side of the tube, and it must not remain long in the fluid.

(*c*) The urinometer must be standardized for the chloroform-benzol mixture. Most urinometers give a reading two or three degrees too high, owing to the low surface tension. Make a mixture such that a drop of distilled water will remain suspended in it (*i. e.*, with a specific gravity of 1.000) and correct the urinometer by this.

(5) **Tallquist Method.**—The popular Tallquist hemoglobinometer consists simply of a book of small sheets of absorbent paper and a carefully printed scale of colors (Fig. 72).

Take up a large drop of blood with the absorbent paper, and when the humid gloss is leaving, before the air has darkened the hemoglobin, compare the stain with the color scale. The color which it matches gives the percentage of hemoglobin. Except in practised hands, this method is accurate only to within 10 or 20 per cent.

Of the methods given, the physician should select the one which best meets his needs. With any method, practice is essential to accuracy. The von Fleischl has long been the standard instrument, but has lately fallen into some disfavor. For accurate work the best instruments are the von Fleischl-Miescher and the Dare. They are, however, expensive, and it is doubtful whether they are enough more accurate than the Sahli instrument to justify the difference in cost. The latter is probably the most satisfactory for the practitioner, provided a well-standardized color-tube is obtained. The specific gravity method is very useful when special instruments are not at hand. The Tallquist scale is so inexpensive and so

convenient that it should be used by every physician at the bedside and in hurried office work; but it should not supersede the more accurate methods.

II. ENUMERATION OF ERYTHROCYTES

In health there are about 5,000,000 red corpuscles per cubic millimeter of blood. Normal variations are slight. The number is generally a little less—about 4,500,000—in women.

Increase of red corpuscles, or *polycythemia*, is unimportant. There is a decided increase following change of residence from a lower to a higher altitude, averaging about 50,000 corpuscles for each 1000 feet, but frequently much greater. The increase, however, is not permanent. In a few months the erythrocytes return to nearly their original number. Three views are offered in explanation: (*a*) Concentration of the blood, owing to increased evaporation from the skin; (*b*) stagnation of corpuscles in the peripheral vessels because of lowered blood-pressure; (*c*) new formation of corpuscles, this giving a compensatory increase of aëration surface.

Pathologically, polycythemia is uncommon. It may occur in: (*a*) Concentration of the blood from severe watery diarrhea; (*b*) chronic heart disease, especially the congenital variety, with poor compensation and cyanosis; and (*c*) idiopathic polycythemia, which is considered to be an independent disease, and is characterized by cyanosis, blood counts of 7,000,000 to 10,000,000, hemoglobin 120 to 150 per cent., and a normal number of leukocytes.

Decrease of red corpuscles, or *oligocythemia*. Red corpuscles and hemoglobin are commonly decreased together, although usually not to the same extent.

Oligocythemia occurs in all but the mildest symptomatic anemias. The blood-count varies from near the normal in moderate cases down to 1,500,000 in very severe cases. There is always a decrease of red cells in chlorosis, but it is often slight, and is relatively less than the decrease of hemoglobin. Leukemia gives a decided oligocythemia, the average count being about 3,000,000. The greatest loss of red cells occurs in pernicious anemia, where counts below 1,000,000 are not uncommon.
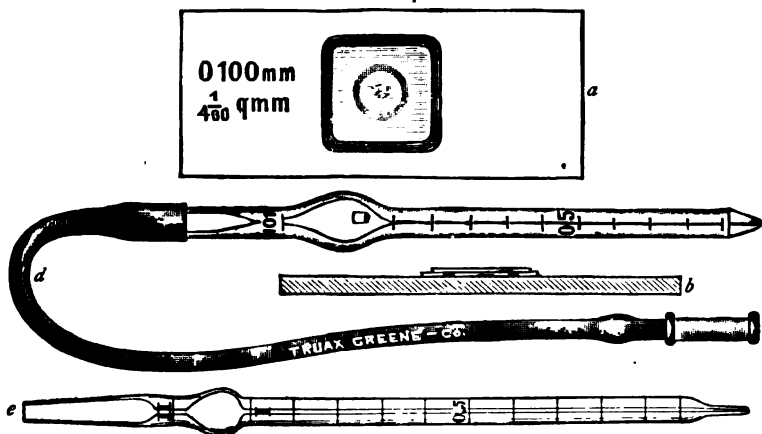
Fig. 73.—Thoma-Zeiss hemocytometer: *a*, Slide used in counting; *b*, sectional view; *d*, red pipet; *e*, white pipet.

The most widely used and most satisfactory instrument for counting the corpuscles is that of Thoma-Zeiss. The hematocrit is not to be recommended for accuracy, since in anemia, where blood-counts are most important, the red cells vary greatly in size and probably also in elasticity. The hematocrit is, however, useful in determining the relative volume of corpuscles and plasma (Volume Index, p. 200), and seems to be gaining in favor.

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The **Thoma-Zeiss instrument** consists of two pipets for diluting the blood and a counting chamber (Fig. 73). The counting chamber is a glass slide with a square platform in the middle. In the center of the platform is a circular opening, in which is set a small circular disc in such a manner that it is surrounded by a " ditch," and that its surface is
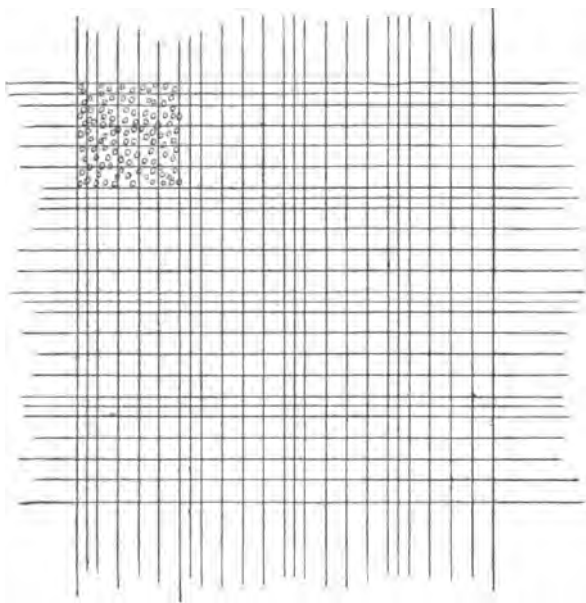
Fig. 74.—Ordinary ruling of counting chamber, showing red corpuscles in left upper corner.

exactly one-tenth of a millimeter below the surface of the square platform. Upon this disc is ruled a square millimeter, subdivided into 400 small squares. Each fifth row of small squares has double rulings for convenience in counting (Fig. 74). A thick cover-glass, ground perfectly plane, accompanies the counting chamber. Ordinary cover-glasses are of uneven surface, and should not be used with this instrument.

It is evident that, when the cover-glass is in place upon the platform, there is a space exactly one-tenth of a millimeter thick between it and the disc; and that, therefore, the square millimeter ruled upon the disc forms the base of a space holding exactly one-tenth of a cubic millimeter.

Technic.—To count the red corpuscles, use the pipet with 101 engraved above the bulb. It must be clean and dry. Obtain a drop of blood as already described. Suck blood

Fig. 75.—Method of drawing blood into the pipet (Boston),

into the pipet to the mark 0.5 or 1. Should the blood go beyond the mark, draw it back by touching the tip of the pipet to a moistened handkerchief. Quickly wipe off the blood adhering to the tip, plunge it into the diluting fluid, and suck the fluid up to the mark 101, slightly rotating the pipet meanwhile. This dilutes the blood 1 : 200 or 1 : 100, according to the amount of blood taken. Except in cases of severe anemia, a dilution of 1 : 200 is preferable. Close the ends

of the pipet with the fingers, and shake vigorously until the blood and diluting fluid are well mixed.

When it is not convenient to count the corpuscles at once, place a heavy rubber band around the pipet so as to close the ends, inserting a small piece of rubber-cloth or other tough, non-absorbent material, if necessary, to prevent the tip from punching through the rubber. It may be kept thus for twenty-four hours or longer.

When ready to make the count, clean the counting chamber and cover-glass, and place a sheet of paper over them to keep off dust. Mix the fluid thoroughly by shaking; blow two or three drops from the pipet, wipe off its tip, and then place a small drop (the proper size can be learned only by experience) upon the disc of the counting chamber. Adjust the cover immediately. Hold it by diagonal corners above the drop of fluid so that a third corner touches the slide and rests upon the edge of the platform. Place a finger upon this corner, and, by raising the finger, allow the cover to fall quickly into place. If the cover be properly adjusted, faint concentric lines of the prismatic colors—Newton's rings—can be seen between it and the platform when the slide is viewed obliquely. They indicate that the two surfaces are in close apposition. If they do not appear at once, slight pressure upon the cover may bring them out. Failure to obtain them is usually due to dirty slide or cover—both must be perfectly clean and *free from dust.* The drop placed upon the disc must be of such size that, when the cover is adjusted, it nearly or quite covers the disc, and that none of it runs over into the " ditch." There should be no bubbles upon the ruled area.

Allow the corpuscles to settle for a few minutes, and then examine with a low power to see that they are evenly distributed. If they are not *evenly distributed over the whole disc,* the counting chamber must be cleaned and a new drop placed in it.

Probably the most satisfactory objective for counting is the special 4 mm. with long working distance. To understand the principle of counting, it is necessary to remember that the large square (400 small squares) represents a capacity of one-tenth of a cubic millimeter. Find the number of corpuscles in the large square, multiply by 10 to find the
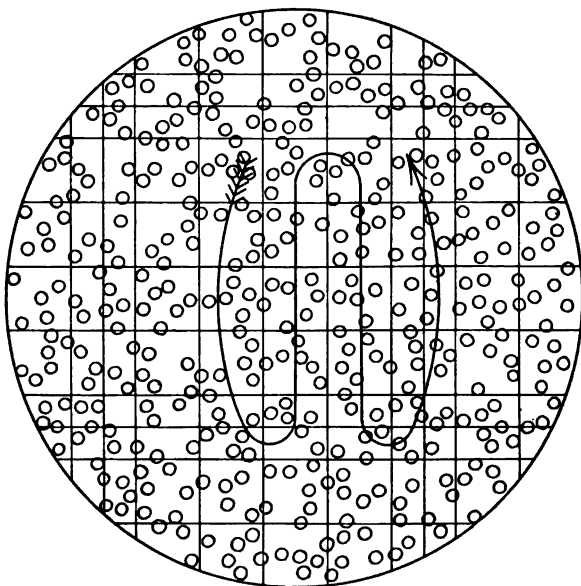
Fig. 76.—Appearance of microscopic field in counting red corpuscles. The arrow indicates the squares to be counted.

number in 1 c.mm. of the diluted blood, and finally, by the dilution, to find the number in 1 c.mm. of undiluted blood. Instead of actually counting all the corpuscles, it is customary to count those in only a limited number of small squares, and from this to calculate the number in the large square. Nearly every worker has his own method of doing this. The essential thing is to adopt a method and adhere to it.

In practice a convenient procedure is as follows: *With a dilution of* 1 : 200, *count the cells in 80 small squares, and to the sum add 4 ciphers; with dilution of* 1 : 100, *count 40 small squares and add 4 ciphers.* Thus, if with 1 : 200 dilution, 450 corpuscles were counted in 80 squares, the total count would be 4,500,000 per c.mm. This method is sufficiently accurate for all clinical purposes, provided the corpuscles are evenly distributed and three drops from the pipet be counted. It is convenient to count a block of 20 small squares, as indicated in Fig. 76, in each corner of the large square. Four columns of 5 squares each are counted. The double rulings show when the bottom of a column has been reached and also indicate the fourth column. In the writer's opinion it is easier to count in vertical than horizontal rows. If distribution be even, the difference between the number of cells in any two such blocks should not exceed twenty. In order to avoid confusion in counting cells which lie upon the border-lines, the following rule is generally adopted: *Corpuscles which touch the upper and left sides should be counted as if within the squares, those touching the lower and right sides, as outside; and vice versâ.*

Diluting Fluids.—The most widely used are Hayem's and Toisson's. Both of these have high specific gravities, so that, when well mixed, the corpuscles do not separate quickly. Toisson's fluid is probably the better for beginners, because it is colored and can easily be seen as it is drawn into the pipet. It stains the nuclei of leukocytes blue, but this is no real advantage. It must be filtered frequently.

HAYEM'S FLUID.		TOISSON'S FLUID.	
Mercuric chlorid	0.5	Methyl-violet, 5 B	0.025
Sodium sulphate	5.0	Sodium chlorid	1.000
Sodium chlorid	1.0	Sodium sulphate	8.000
Distilled water	200.0	Glycerin	30.000
		Distilled water	160.000

Sources of Error.—The most common sources of error in making a blood count are:

(*a*) Inaccurate dilution, either from faulty technic or inaccurately graduated pipets. The instruments made by Zeiss can be relied upon.

(*b*) Too slow manipulation, allowing a little of the blood to coagulate and remain in the capillary portion of the pipet.

(*c*) Inaccuracy in depth of counting chamber, which sometimes results from softening of the cement by alcohol or heat. The slide should not be cleaned with alcohol nor left to lie in the warm sunshine.

(*d*) Uneven distribution of the corpuscles. This results when the blood is not thoroughly mixed with the diluting fluid, or when the cover-glass is not applied soon enough after the drop is placed upon the disc.

Cleaning the Instrument.—The instrument should be cleaned immediately after using, and the counting chamber and cover must be cleaned again just before use.

Draw through the pipet, successively, water, alcohol, ether, and air. This can be done with the mouth, but it is much better to use a rubber bulb or suction filter pump. When the mouth is used, the moisture of the breath will condense upon the interior of the pipet unless the fluids be shaken and not blown out. If blood has coagulated in the pipet—which happens when the work is done too slowly—dislodge the clot with a horsehair, and clean with strong sulphuric acid, or let the pipet stand over night in a test-tube of the acid. Even if the pipet does not become clogged, it should be occasionally cleaned in this way. When the etched graduations on the pipets become dim, they can be renewed by rubbing with a grease pencil.

Wash the counting-chamber and the cover with water and dry them with clean soft linen. Alcohol may be used to clean the latter, but never the former.

III. COLOR INDEX

This is an expression which indicates the amount of hemoglobin in each red corpuscle compared with the normal amount. For example, a color index of 1.0 indicates that each corpuscle contains the normal amount of hemoglobin; of 0.5, that each contains one-half the normal.

The color index is most significant in chlorosis and pernicious anemia. In the former it is usually much decreased; in the latter, generally much increased. In symptomatic anemia it is generally moderately diminished.

To obtain the color index, divide the percentage of hemoglobin by the percentage of corpuscles. The percentage of corpuscles is found by multiplying the first two figures of the red corpuscle count by two. This simple method holds good for all counts of 1,000,000 or more. Thus, a count of 2,500,000 is 50 per cent. of the normal. If, then, the hemoglobin has been estimated at 40 per cent., divide 40 (the percentage of hemoglobin) by 50 (the percentage of corpuscles). This gives $\frac{4}{5}$, or 0.8, as the color index.

IV. VOLUME INDEX

The term " volume index " was introduced by Capps to express the average size of the red cells of an individual compared with their normal size. It is the quotient obtained by dividing the *volume* of red corpuscles (expressed in percentage of the normal) by the *number* of red corpuscles, also expressed in percentage of the normal.

The volume index more or less closely parallels the color index, and variations have much the same sig-

nificance. The following are averages of the examinations reported by Larrabee in the Journal of Medical Research:

	Red corpuscles per cubic millimeter.	Hemoglobin per cent. by Sahli instrument.	Color index.	Volume index.
Normal males	5,267,250	103.0	0.98	1.007
Normal females...........	4,968,667	106.0	1.06	1.001
Primary pernicious anemia.	1,712,166	50.0	1.47	1.270
Secondary anemia.........	3,737,160	61.0	0.81	0.790
Chlorosis................	3,205,000	34.5	0.55	0.695

Method.—The red cells are counted and the percentage of red cells calculated as for the color index.

The volume percentage is obtained with the hematocrit as follows: Fill the hematocrit tubes (Fig. 77) with blood, and before coagulation takes place insert them in the frame and centrifugalize for three minutes at about 8000 to 10,000 revolutions a minute. The red cells collect at the bottom and, normally, make up one-half of the total column of blood.
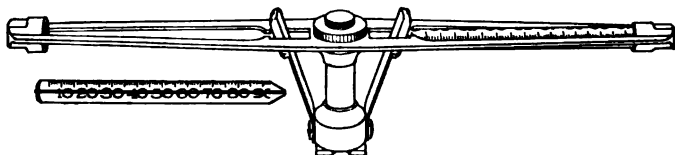
Fig. 77.—Daland hematocrit for use with the centrifuge.

Multiply the height of the layer of red cells (as indicated by the graduations upon the side of the tube) by 2 to obtain the volume percentage. When the examination cannot be made immediately after the blood is obtained, the method of Larrabee is available. This consists in mixing a trace of sodium oxalate with a few drops of blood to prevent coagulation, drawing this mixture into a tube of about 2-mm. caliber and waiting until sedimentation is complete—usually about three days. The height of the column is then measured with a

millimeter scale and the percentage relation to the normal calculated.

After the volume of the red cells and the red corpuscle count are thus expressed in percentages, divide the former by the latter to find the volume index. Example: Suppose the volume percentage is 80 (the reds reaching to mark 40 on hematocrit tube) and that the red count is 50 per cent. of the normal (2,500,000 per c.mm.), then $\frac{80}{50}$ or 1.6 is the volume index.

V. ENUMERATION OF LEUKOCYTES

The normal number of leukocytes varies from 5000 to 10,000 per cubic millimeter of blood. The number is larger in robust individuals than in poorly nourished ones, and if disease be excluded, may be taken as a rough index of the individual's nutrition. Since it is well to have a definite standard, 7500 is generally adopted as the normal for the adult. With children the number is somewhat greater, counts of 12,000 and 15,000 being common in healthy children under twelve years of age.

DECREASE IN NUMBER OF LEUKOCYTES

Decrease in number of leukocytes, or *leukopenia*, is not important. It is common in persons who are poorly nourished, although not actually sick. The infectious diseases in which leukocytosis is absent (p. 206) often cause a slight decrease of leukocytes. Chlorosis may produce leukopenia, as also pernicious anemia, which usually gives it in contrast to the secondary anemias, which are frequently accompanied by leukocytosis. Leukocyte counts are, therefore, of some aid in the differential diagnosis of these conditions.

INCREASE IN NUMBER OF LEUKOCYTES

Increase in number of leukocytes is common and of great importance. It may be considered under two heads:

A. Increase of leukocytes due to chemotaxis and stimulation of the blood-making organs, or *leukocytosis.* The increase affects one or more of the normal varieties.

B. Increase of leukocytes due to *leukemia.* Normal varieties are increased, but the characteristic feature is the appearance of great numbers of abnormal cells.

The former may be classed as a *transient*, the latter, as a *permanent*, increase.

A. LEUKOCYTOSIS

This term is variously used. By some it is applied to any increase in number of leukocytes; by others it is restricted to increase of the polymorphonuclear neutrophilic variety. As has been indicated, it is here taken to mean a transient increase in number of leukocytes, that is, one caused by chemotaxis and stimulation of the blood-producing structures, in contrast to the permanent increase caused by leukemia.

By *chemotaxis* is meant that property of certain agents by which they attract or repel living cells—positive chemotaxis and negative chemotaxis respectively. An excellent illustration is the accumulation of leukocytes at the site of inflammation, owing to the positively chemotactic influence of bacteria and their products. A great many agents possess the power of attracting leukocytes into the general circulation. Among these are many bacteria and certain organic and inorganic poisons.

Chemotaxis alone will not explain the continuance of

leukocytosis for more than a short time. It is probable that substances which are positively chemotactic also stimulate the blood-producing organs to increased formation of leukocytes; and in at least one form of leukocytosis such stimulation apparently plays the chief part.

As will be seen later, there are several varieties of leukocytes in normal blood, and most chemotactic agents attract only one variety, and either repel or do not influence the others. It practically never happens that all are increased in the same proportion. The most satisfactory classification of leukocytoses is, therefore, based upon the type of leukocyte chiefly affected.

Theoretically, there should be a subdivision for each variety of leukocyte, *e. g.*, polymorphonuclear leukocytosis, lymphocyte leukocytosis, eosinophilic leukocytosis, large mononuclear leukocytosis, etc. Practically, however, only two of these, polymorphonuclear leukocytosis and lymphocyte leukocytosis, need be considered under the head of Leukocytosis. Increase in number of the other leukocytes will be considered when the individual cells are described (pp. 230–243). They are present in the blood in such small numbers normally that even a marked increase scarcely affects the total leukocyte count; and, besides, substances which attract them into the circulation frequently repel the polymorphonuclears, so that the total number of leukocytes may actually be decreased.

The polymorphonuclear neutrophiles are capable of active ameboid motion, and are by far the most numerous of the leukocytes. Lymphocytes are about one-third as numerous and have little independent motion. As one would, therefore, expect, marked differences exist

between the two types of leukocytosis: polynuclear leukocytosis is more or less acute, coming on quickly and often reaching high degree; whereas lymphocyte leukocytosis is more chronic, comes on more slowly, and is never so marked.

1. Polymorphonuclear Neutrophilic Leukocytosis.— Polymorphonuclear leukocytosis may be either physiologic or pathologic. A count of 20,000 would be considered a marked leukocytosis; of 30,000, high; above 50,000, extremely high.

(1) **Physiologic Polymorphonuclear Leukocytosis.—** This is never very marked, the count rarely exceeding 15,000 per cubic millimeter. It occurs: (*a*) In the newborn; (*b*) in pregnancy; (*c*) during digestion, and (*d*) after cold baths. There is moderate leukocytosis in the moribund state: this is commonly classed as physiologic, but is probably due mainly to terminal infection.

The increase in these conditions is not limited to the polymorphonuclears. Lymphocytes are likewise increased in varying degrees, most markedly in the newborn.

In view of the leukocytosis of digestion, the hour at which a leukocyte count is made should always be recorded. Digestive leukocytosis is most marked three to five hours after a hearty meal rich in protein. It is absent in pregnancy and when leukocytosis from any other cause exists. It is usually absent in cancer of the stomach, a fact which may be of some help in the diagnosis of this condition, but repeated examinations are essential.

(2) **Pathologic Polymorphonuclear Leukocytosis.—** In general, the response of the leukocytes to chemotaxis

is a conservative process. It has been compared to the gathering of soldiers to destroy an invader. This is accomplished partly by means of phagocytosis—actual ingestion of the enemy—and partly by means of chemic substances which the leukocytes produce.

In those diseases in which leukocytosis is the rule the degree of leukocytosis depends upon two factors: the *severity of the infection* and the *resistance of the individual.* A well-marked leukocytosis usually indicates good resistance. A mild degree means that the body is not reacting well, or else that the infection is too slight to call forth much resistance. Leukocytosis may be absent altogether when the infection is extremely mild, or when it is so severe as to overwhelm the organism before it can react. When leukocytosis is marked, a sudden fall in the count may be the first warning of a fatal issue. These facts are especially true of pneumonia, diphtheria, and abdominal inflammations, in which conditions the degree of leukocytosis is of considerable prognostic value.

The classification here given follows Cabot:

(*a*) *Infectious and Inflammatory.*—The majority of infectious diseases produce leukocytosis. The most notable exceptions are influenza, malaria, measles, tuberculosis, except when invading the serous cavities or when complicated by mixed infection, and typhoid fever, in which leukocytosis indicates an inflammatory complication.

All inflammatory and suppurative diseases cause leukocytosis, except when slight or well walled off. Appendicitis has been studied with especial care in this connection, and the conclusions now generally accepted probably hold good for most acute intra-abdominal inflam-

mations. A marked leukocytosis (20,000 or more) nearly always indicates abscess, peritonitis, or gangrene, even though the clinical signs be slight. Absence of or mild leukocytosis indicates a mild process, or else an overwhelmingly severe one; and operation may safely be postponed unless the abdominal signs are very marked. On the other hand, no matter how low the count, an increasing leukocytosis—counts being made hourly—indicates a spreading process and demands operation, regardless of other symptoms.

Leukocyte counts alone are often disappointing, but are of much more value when considered in connection with a differential count of polymorphonuclears. (See p. 236.)

(*b*) *Malignant Disease.*—Leukocytosis occurs in about one-half of the cases of malignant disease. In many instances it is probably independent of any secondary infection, since it occurs in both ulcerative and non-ulcerative cases. It seems to be more common in sarcoma than in carcinoma. Very large counts are rarely noted.

(*c*) *Posthemorrhagic.*—Moderate leukocytosis follows hemorrhage and disappears in a few days.

(*d*) *Toxic.*—This is a rather obscure class, which includes gout, chronic nephritis, acute yellow atrophy of the liver, ptomain-poisoning, prolonged chloroform narcosis, and quinin-poisoning. Leukocytosis may or may not occur in these conditions, and is not important.

(*e*) *Drugs.*—This also is an unimportant class. Most tonics and stomachics and many other drugs produce a slight leukocytosis.

2. Lymphocyte Leukocytosis.—This is characterized by an increase in the total leukocyte count, accom-

panied by an increase in the percentage of lymphocytes. The word " lymphocytosis " is often used in the same sense. It is better, however, to use the latter as referring to any increase in the absolute number of lymphocytes, without regard to the total count, since an absolute increase in number of lymphocytes is frequently accompanied by a normal or subnormal leukocyte count, owing to loss of polymorphonuclears.

Non-phagocytic leukocytosis is probably due more to stimulation of blood-making organs than to chemotaxis. It is less common, and is rarely so marked as a polymorphonuclear leukocytosis. When marked, the blood cannot be distinguished from that of lymphatic leukemia.

A marked lymphocyte leukocytosis occurs in pertussis, and is of value in diagnosis. It appears early in the catarrhal stage, and persists until after convalescence. The average leukocyte count is about 17,000, lymphocytes predominating. There is moderate lymphocyte leukocytosis in other diseases of childhood, as rickets, scurvy, and especially hereditary syphilis, where the blood-picture may approach that of pertussis. It must be borne in mind in this connection that lymphocytes are normally more abundant in the blood of children than in that of adults.

Slight lymphocyte leukocytosis occurs in many other pathologic conditions, but is of little significance.

B. Leukemia

This is an idiopathic disease of the blood-making organs, which is accompanied by an enormous increase in number of leukocytes. The leukocyte count sometimes reaches 1,000,000 per cubic millimeter, and leu-

kemia is always to be suspected when it exceeds 50,000. Lower counts do not, however, exclude it. The subject is more fully discussed later (p. 280).

METHOD OF COUNTING LEUKOCYTES

The leukocytes are counted with the Thoma-Zeiss instrument, already described. Recently, several new rulings of the disc have been introduced, notably the Zappert and the Türck (Fig. 79), which give a ruled area of nine square millimeters. They were devised for counting the leukocytes in the same specimen with the red corpuscles. The red cells are counted in the usual manner, after which all the leukocytes in the whole area of nine square millimeters are counted; and the number in a cubic millimeter of undiluted blood is then easily calculated. Leukocytes are easily distinguished from red cells, especially when Toisson's diluting fluid is used. This method may be used with the ordinary ruling by adjusting the microscopic field to a definite size, and counting a sufficient number of fields, as described later. Although less convenient, it is more accurate to count the leukocytes separately, with less dilution of the blood, as follows:

Technic.—A larger drop of blood is required than for counting the erythrocytes, and more care in filling the pipet. Boggs has suggested a device (Fig. 78) which enables one to draw in the blood more slowly and hence more accurately. He cuts the rubber tube and inserts a Wright " throttle." This consists of a section of glass tubing in which a capillary tube drawn out to a fine thread is cemented with sealing wax. After sealing in place the tip is broken off with forceps, so that upon gentle suction it will just allow air to pass.

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Use the pipet with 11 engraved above the bulb. Suck the blood to the mark 0.5 or 1.0, and the diluting fluid to the mark 11. This gives a dilution of 1:20 or 1:10, respectively. The dilution of 1 : 20 is easier to make. Mix well by shaking in all directions except in the long axis of the pipet; blow out two or three drops, place a drop in the counting chamber, and adjust the cover as already described (p. 196).
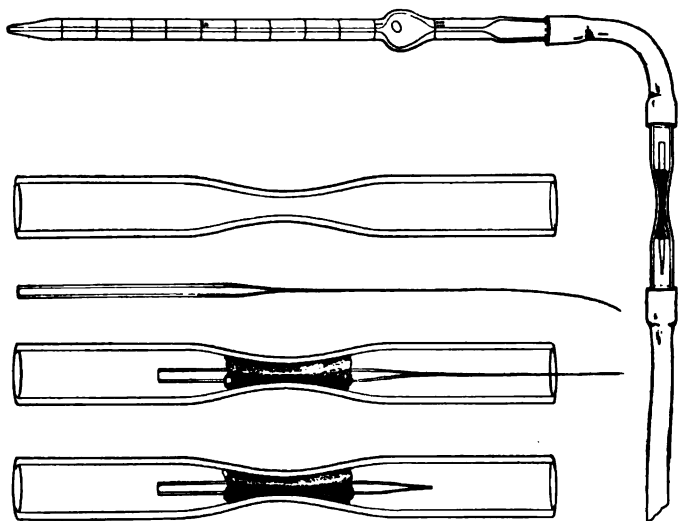
Fig. 78.—Boggs' "throttle control" for blood-counting pipet, and enlarged diagram showing construction of the throttle.

Examine with a low power to see that the cells are evenly distributed. Count with the 16 mm. objective and a high eye-piece, or with the long-focus 4 mm. and a low eye-piece. An 8 mm. objective will be found very satisfactory for this purpose. As one gains experience one will rely more upon the lower powers.

With the ordinary ruling of the disc, count all the leuko-cytes in the large square, multiply by 10 to find the number in

1 c.mm. of diluted blood, and by the dilution to find the number per c.mm. of undiluted blood. In every case at least 200 leukocytes must be counted as a basis for calculation, and it is much better to count 500. This will necessitate examination of several drops from the pipet. With the Zappert
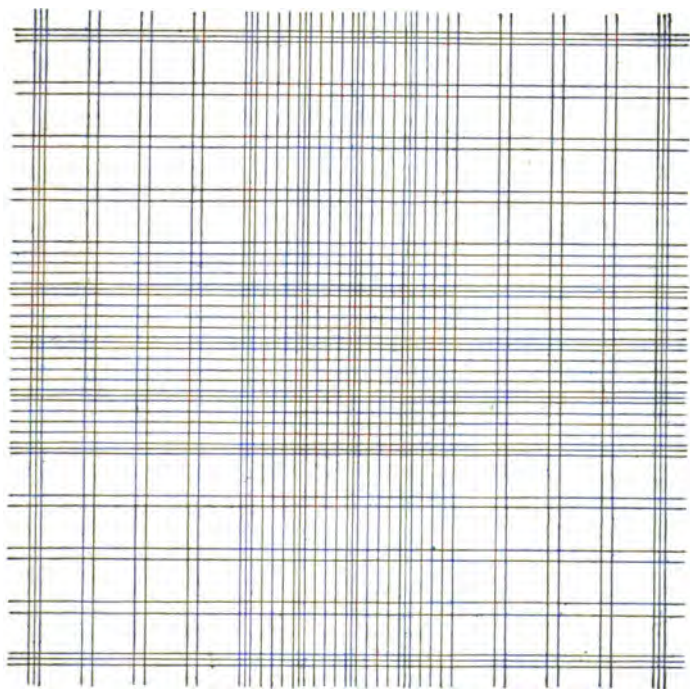
Fig. 79.—Türck ruling of counting chamber.

and Türck rulings a sufficient number can usually be counted in one drop, but the opportunity for error is very much greater when only one drop is examined.

In routine work the author's modification of the "circle" method is very satisfactory. It requires a 4 mm. objective,

and is, therefore, especially desirable for beginners, who are usually unable accurately to identify leukocytes with a lower power. The student is frequently confused by particles of dirt, remains of red cells, and yeast cells which sometimes grow in the diluting fluid. Draw out the sliding tube of the
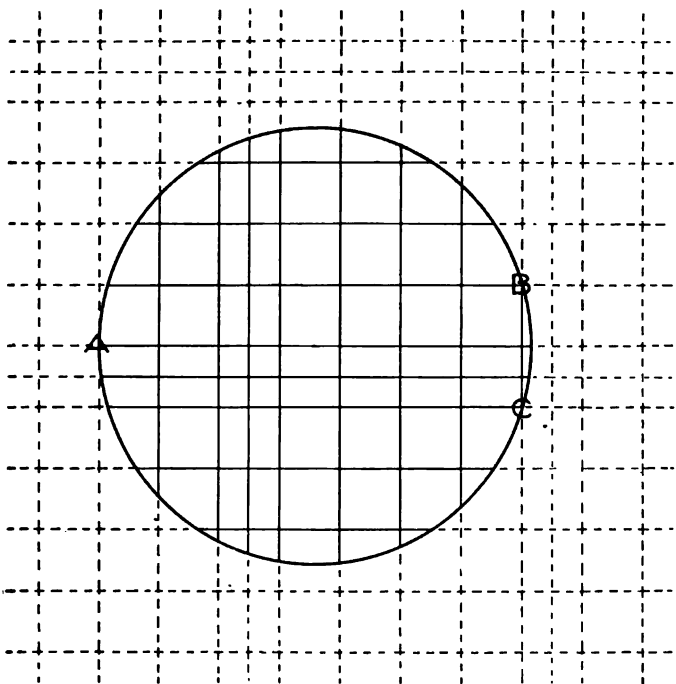
Fig. 80.—Size of field required in counting leukocytes as described in the text.

microscope until the field of vision is such as shown in Fig. 80. One side of the field is tangent to one of the ruled lines, A, while the opposite side just cuts the corners, B and C, of the seventh squares in the rows above and below the diameter line. When once adjusted, a scratch is made upon the draw-

tube, so that for future counts the tube has only to be drawn out to the mark. The area of this microscopic field is one-tenth of a square millimeter. With a dilution of 1 : 20, count the leukocytes in 20 such fields upon different parts of the disc without regard to the ruled lines, and to their sum add two ciphers. With dilution of 1 : 10, count 10 such fields, and add two ciphers. Thus, with 1 : 10 dilution, if 150 leukocytes were counted in 10 fields, the leukocyte count would be 15,000 per c.mm. To compensate for possible unevenness of distribution, it is best to count a row of fields horizontally and a row vertically across the disc. This method is applicable to any degree of dilution of the blood, and is simple to remember: *one always counts a number of fields equal to the number of times the blood has been diluted, and adds two ciphers.*

It is sometimes impossible to obtain the proper size of field with the objectives and eye-pieces at hand. In such case place a cardboard disc with a circular opening upon the diaphragm of the eye-piece, and adjust the size of the field by drawing out the tube. The circular opening can be cut with a cork-borer.

Diluting Fluids.—The diluting fluid should dissolve the red corpuscles so that they will not obscure the leukocytes. The simplest fluid is a 0.5 per cent. solution of acetic acid. More satisfactory is the following: glacial acetic acid, 1 c.c.; 1 per cent. aqueous solution of gentian-violet, 1 c.c.; distilled water, 100 c.c. These solutions must be filtered frequently.

VI. ENUMERATION OF BLOOD-PLAQUES

The average normal number of plaques is variously given as 200,000 to 700,000 per cubic millimeter of blood. Many of the counts were obtained by workers who used unreliable methods. Using their new method, Wright and Kinnicutt find the normal average to range from 263,000 to 336,000. Physiologic variations are

marked; thus, the number increases as one ascends to a higher altitude, and is higher in winter than in summer. There are unexplained variations from day to day; hence a single abnormal count should not be taken to indicate a pathologic condition.

Pathologic variations are often very great. Owing to lack of knowledge as to the origin of the platelets and to the earlier imperfect methods of counting, the clinical significance of these variations is uncertain. The following facts seem, however, to be established:

(a) In acute infectious diseases the number is subnormal or normal. If the fever ends by crisis, the crisis is accompanied by a rapid and striking increase.

(b) In secondary anemia plaques are generally increased, although sometimes decreased In pernicious anemia they are always greatly diminished, and an increase should exclude the diagnosis of this disease.

(c) They are decreased in chronic lymphatic leukemia, and greatly increased in the myelogenous form.

(d) In purpura hæmorrhagica the number is enormously diminished.

Blood-plaques are difficult to count, owing to the rapidity with which they disintegrate and to their great tendency to adhere to any foreign body and to each other.

Method of Kemp, Calhoun, and Harris.—Wash the finger well and allow a few minutes to elapse for the circulation to become normal. Prick the finger lightly with a blood-lancet, regulating the depth of the puncture so that the blood will not flow without gentle pressure. Quickly dip a clean glass rod into a vessel containing diluting and fixing fluid, and place two or three good-sized drops upon the

finger over the puncture.   Then exert gentle pressure above
the puncture so that a small drop of blood will exude into the
fluid.   Mix the two by passing the rod lightly several times
over the surface of the blended drop.   (Some workers first
place a drop of the fluid upon the finger and then make the
puncture through it, this necessitating less care as to depth of
the puncture.)   Now transfer a drop of the diluted blood from
the finger to a watch-glass which contains two or three drops
of the fluid, and mix well.   From this, transfer a drop to the
counting slide of the Thoma-Zeiss hemocytometer, and cover.
An ordinary thin cover will answer for this purpose, and is
preferable because it allows the use of a higher power object-
ive.   Allow the slide to stand for at least five minutes, and
then with a 4 mm. or higher objective count the plaques
and the red corpuscles in a definite number of squares.   At
least 100 plaques must be counted.   The number of red cor-
puscles per cubic millimeter of blood having been previously
ascertained in the usual manner (p. 195), the number of
plaques can easily be calculated by the following equation:

$$r : p : : R : P; \text{ and } P = \frac{p \times R}{r}.$$

r represents the number of red corpuscles in any given
number of squares; p, the number of plaques in the same
squares; R, the total number of red corpuscles per c.mm. of
blood; and P, the number of plaques per c.mm.

Beginners are apt to take too much blood and not to dilute
it enough.   Best results are attained when there are only one
or two plaques in a small square.   With insufficient dilution,
the platelets are more or less obscured by the red cells.

The following diluting and fixing fluid is recommended:

Formalin................................... 10 c.c.
1 per cent. aqueous solution sodium chlorid...... 150 c.c.
(Color with methyl-violet if desired.)

This fluid is cheap and easily prepared, and keeps indefinitely. It fixes the plaques quickly without clumping, and does not clump nor decolorize the reds. It has a low specific gravity, and hence allows the plaques to settle upon the ruled area along with the reds. Fluids of high specific gravity cause the plaques to float so that they do not appear in the same plane with the reds and the ruled lines.

Method of Wright and Kinnicutt.—This new method is simple, appears to be accurate, and certainly yields uniform results.

The plaques are counted with the Thoma-Zeiss hemocytometer already described, using a dilution of 1 : 100. The diluting fluid consists of two parts of an aqueous solution of " brilliant cresyl blue " (1 : 300) and three parts of an aqueous solution of potassium cyanid (1 : 1400). These two solutions must be kept in separate bottles and mixed and filtered immediately before using. After the blood is placed in the counting-chamber it is allowed to stand for ten minutes or longer in order that the plaques may settle. The plaques appear as rounded, lilac-colored bodies; the reds are decolorized, appearing only as shadows.

The leukocytes are stained and may be counted at the same time.

VII. STUDY OF STAINED BLOOD

A. MAKING AND STAINING BLOOD-FILMS

1. Spreading the Film.—Thin, even films are essential to accurate and pleasant work. They more than compensate for the time spent in learning to make them. There are certain requisites for success with any method: (*a*) The slides and covers must be perfectly clean: thorough washing with soap and water and rubbing with alcohol will usually suffice; (*b*) the drop of blood

must not be too large; (*c*) the work must be done quickly, before coagulation begins.

The blood is obtained from the finger-tip or the lobe of the ear, as for a blood count; only a very small drop is required.

Ehrlich's Two Cover-glass Method.—This method is very widely used, but considerable practice is required to get good results. Touch a cover-glass to the top of a small drop of blood, and place it, blood side down, upon another cover-
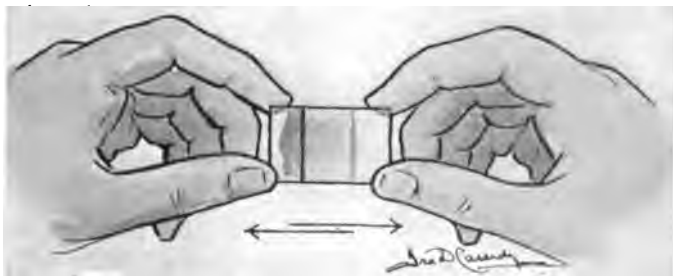
Fig. 81.—Spreading the film: two cover-glass method.

glass. If the drop be not too large, and the covers be perfectly clean, the blood will spread out in a very thin layer. Just as it stops spreading, before it begins to coagulate, pull the covers quickly but firmly apart on a line parallel to their plane (Fig. 81). It is best to handle the covers with forceps, since the moisture of the fingers may produce artifacts.

Two-slide Method.—Take a small drop of blood upon a clean slide about ½ inch from the end. Place the end of a second slide against the surface of the first at an angle of 45°, and push it up against the drop of blood, which will immediately run across the end, filling the angle between the

two slides. Now draw the " spreader slide " back along the other. The blood will follow. The thickness of the smear can be regulated by changing the angle.
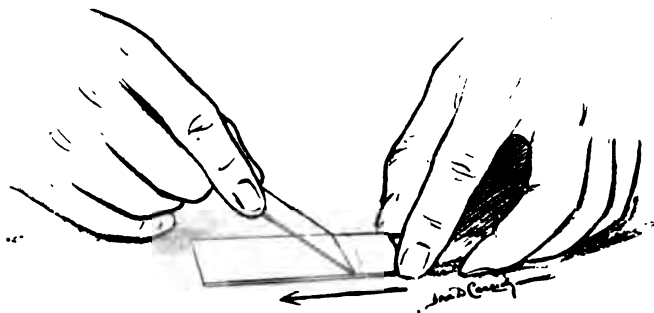
Fig. 82.—Spreading the film: two-slide method.

Cigarette-paper Method.—This gives better results in the hands of the inexperienced than any of the methods in general
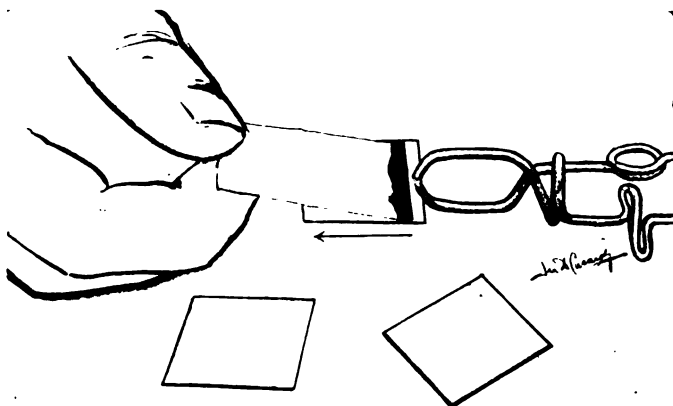
Fig. 83.—Spreading the film. Cigarette-paper method applied to cover-glasses.

use, and may be used with either slides or covers. A very thin paper, such as the " Zig-zag " brand, is best. Ordinary

cigarette paper and thin tissue-paper will answer, but do not give nearly so good results.

Cut the paper into strips about ¾ inch wide, *across the ribs*. Pick up one of the strips by the gummed edge, and touch its opposite end to the drop of blood. Quickly place the end which has the blood against a slide or a large cover-glass held in a forceps. The blood will spread along the edge of the paper. Now draw the paper evenly across the slide or cover. A thin film of blood will be left behind (Fig. 83).

The films may be allowed to dry in the air, or may be dried by gently heating high above a flame (where one can comfortably hold the hand). Such films will keep for years, but for some stains they must not be more than a few weeks old. They must be kept away from flies—a fly can work havoc with a film in a few minutes.

2. Fixing the Film.—In general, films must be "fixed" before they are stained. Fixation may be accomplished by chemicals or by heat. *Those stains which are dissolved in methyl-alcohol combine fixation with the staining process.*

Chemic Fixation.—Soak the film five to fifteen minutes in pure methyl-alcohol, or one-half hour or longer in equal parts of absolute alcohol and ether. One minute in 1 per cent. formalin in alcohol is preferred by some. Chemic fixation may precede eosin-methylene-blue and other simple stains.

Heat Fixation.—This may precede any of the methods which do not combine fixation with the staining process; it *must* be used with Ehrlich's triple stain. The best method is to place the film in an oven, raise the temperature to 150° C., and allow to cool slowly. Without an oven, the proper degree of fixation is difficult to attain. Kowarsky has devised a small plate of two layers of copper (Fig. 84), upon

which the films are placed together with a crystal of urea. The plate is heated over a flame until the urea melts, and is then set aside to cool. This is said to give the proper degree of fixation, but in the writer's experience the films have always been underheated. He obtains better results by use of tartaric acid crystals (melting-point, 168°–170° C.). The plate, upon which have been placed the cover-glasses, film side down, and a crystal of the acid, is heated over a low flame until the crystal has completely melted. It should be held sufficiently high above the flame that the heating will require five to seven minutes. The covers are then removed. Freshly made films of normal blood should be allowed to remain upon
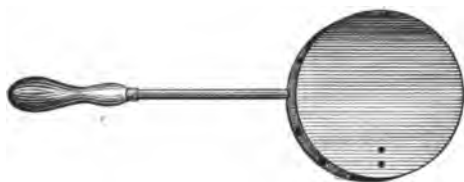
Fig. 84.—Kowarsky's plate for fixing blood (Klopstock and Kowarsky).

the plate for a minute or two after heating has ceased. Fresh films require more heat than old ones, and normal blood more than the blood of pernicious anemia and leukemia.

Fixation by passing the film quickly through a flame about twenty times, as is often done in routine work, is not recommended for beginners.

3. **Staining the Film.**—The anilin dyes, which are extensively used in blood work, are of two general classes: basic dyes, of which methylene-blue is the type; and acid dyes, of which eosin is the type. Nuclei and certain other structures in the blood are stained by the basic dyes, and are hence called *basophilic*. Certain struc-

tures take up only acid dyes, and are called *acidophilic*, *oxyphilic*, or *eosinophilic*. Certain other structures are stained only by combinations of the two, and are called *neutrophilic*. Recognition of these staining properties marked the beginning of modern hematology.

(1) **Eosin and Methylene-blue.**—In many instances this stain will give all the information desired. It is especially useful in studying the red corpuscles. Nuclei, basophilic granules, and all blood parasites are blue; erythrocytes are red or pink; eosinophilic granules, bright red. Neutrophilic granules and blood-plaques are not stained.

(1) Fix the film by heat or chemicals.

(2) Stain about five minutes with 0.5 per cent. alcoholic solution of eosin, diluted one-half with water.

(3) Rinse in water, and dry between filter-papers.

(4) Stain one-half to one minute with saturated aqueous solution of methylene-blue.

(5) Rinse well, dry, and mount. Films upon slides may be examined with an oil-immersion objective without a cover-glass.

(2) **Ehrlich's Triple Stain.**—This has been the standard blood-stain for many years, but is now little used. It is probably the best for neutrophilic granules. It is difficult to make, and should be purchased ready prepared from a reliable dealer. Nuclei are stained blue or greenish blue; erythrocytes, orange; neutrophilic granules, violet; and eosinophilic granules, copper red. Basophilic granules and blood-plaques are not stained.

Success in staining depends largely upon proper fixation. The film must be carefully fixed by heat: under-

heating causes the erythrocytes to stain red; overheating, pale yellow. The staining fluid is applied for five to fifteen minutes, and the preparation is rinsed quickly in water, dried, and mounted. Subsequent application of Löffler's methylene-blue for one-half to one second will bring out the basophilic granules and improve the nuclear staining, but there is considerable danger of overstaining.

(3) **Polychrome Methylene-blue Eosin Stains.**—These stains, outgrowths of the original Romanowsky method, have largely displaced other blood-stains for clinical purposes. They stain differentially every normal and abnormal structure in the blood. Most of them are dissolved in methyl alcohol and combine the fixing with the staining process. Numerous methods of preparing and applying these stains have been devised. Two only need be given here: Wright's stain and Harlow's stain:

Wright's Stain.—This is one of the best and is the most widely used in this country. The practitioner will find it best to purchase the stain ready prepared. Most microscopic supply-houses carry it in stock. Wright's most recent directions for its preparation and use are as follows:[1]

Preparation.—To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene-blue (B. X. or " medicinally pure ") in the proportion of 1 gm. of the dye to each 100 c.c. of the solution. Heat the mixture in a steam sterilizer at 100° C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask, or flasks, of such size and shape that it

[1] Journal of the American Medical Association, Dec. 3, 1910.

forms a layer not more than 6 cm. deep. After heating, allow the mixture to cool, placing the flask in cold water, if desired, and then filter it to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed in a thin layer by transmitted yellowish artificial light. It does not show this color while it is warm.

To each 100 c.c. of the filtered mixture add 500 c.c. of a 0.1 per cent. aqueous solution of " yellowish water-soluble " eosin and mix thoroughly. Collect the abundant precipitate which immediately appears on a filter. When the precipitate is dry, dissolve it in methylic alcohol (Merck's " reagent ") in the proportion of 0.1 gm. to 60 c.c. of the alcohol. In order to facilitate solution, the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle. This alcoholic solution of the precipitate is the staining fluid.

Application.—1. Cover the film with a noted quantity of the staining fluid by means of a medicine-dropper.

2. After one minute add to the staining fluid on the film the same quantity of distilled water by means of a medicine-dropper and allow the mixture to remain for two or three minutes, according to the intensity of the staining desired. A longer period of staining may produce a precipitate. Eosinophilic granules are best brought out by a short period of staining.

The quantity of the diluted fluid on the preparation should not be so large that some of it runs off.

3. Wash the preparation in water for thirty seconds or until the thinner portions of the film become yellow or pink in color.

4. Dry and mount in balsam.

The stain is more conveniently applied upon cover-glasses than upon slides. Films much more than a month old do not stain well. In some localities ordinary tap-

water will answer both for diluting the stain and for washing the film; in others, distilled water must be used. Different lots of Wright's fluid vary, and a few preliminary stains should be made with each lot to learn its peculiarities.

When properly applied, Wright's stain gives the following picture (Plate VI): erythrocytes, yellow or pink; nuclei, various shades of bluish purple; neutrophilic granules, reddish lilac; eosinophilic granules, bright red; basophilic granules of leukocytes and degenerated red corpuscles, very dark bluish purple; blood-plaques, dark lilac; bacteria, blue. The cytoplasm of lymphocytes is generally robin's-egg blue; that of the large mononuclears may have a faint bluish tinge. Malarial parasites stain characteristically: the cytoplasm, sky-blue; the chromatin, reddish purple.

Harlow's Stain.—Probably the simplest modification of the Romanowsky stain, both in preparation and method of use, is that devised by W. P. Harlow of the University of Colorado. It differentiates granules particularly well, but is not so satisfactory for demonstrating *slight* grades of polychromatophilia, because it usually gives all the red cells a faint bluish tinge.

Preparation.—The stain consists of two solutions used separately:

No. 1. Eosin, yellowish, water soluble (Grübler). 1 gram
Methyl alcohol (Merck's reagent) . . . . 100 c.c.
No. 2. Methylene-blue (" B. X." or Ehrlich's
rectified) (Grübler) . . . . 1 gram
Methyl alcohol (Merck's reagent) . . . . 100 c.c.

Application.—(1) Stain the film without previous fixation for one minute with the eosin solution.

(2) Shake off the excess, allowing a very little to remain, and at once cover with the methylene-blue solution for one or two minutes.

(3) Rinse quickly in distilled water, dry, and mount.

It is well known that pathologic bloods will sometimes not stain well with fluids which are satisfactory for normal bloods.   Doctors Peebles and Harlow have shown that the various polychrome methylene-blue-eosin stains can be modified to suit any blood by adding a little alkali or acid.   The alkali used is a weak solution of " potassium hydrate by alcohol " in methyl alcohol; the acid, glacial acetic in methyl alcohol.   In the case of the Harlow stain it is added to the methylene-blue solution only.   The alkali solution also serves to " correct " old fluids which, by reason of development of formic acid in the methyl alcohol, do not stain sufficiently with the blue.   In general a stain is satisfactory when both nuclei and neutrophilic granules are clearly defined.

B. ·STUDY OF STAINED FILMS

Much can be learned from stained blood-films.   They furnish the best means of studying the morphology of the blood and blood parasites, and, to the experienced, they give a fair idea of the amount of hemoglobin and the number of red and white corpuscles.   An oil-immersion objective is required.

1. **Erythrocytes.**—Normally, the red corpuscles are acidophilic.   The colors which they take with different stains have been described.   When not crowded together, they appear as circular, homogeneous discs, of nearly uniform size, averaging 7.5μ in diameter (Fig. 104).   The center of each is somewhat paler than the

15

periphery. The degree of pallor furnishes a rough index to the amount of hemoglobin in the corpuscle. As hemoglobin is diminished, the central pale area becomes larger and paler, producing the so-called "pessary forms" in which only the periphery of the cell is apparent. These forms indicate a low color index and are most abundant in chlorosis. Red cells are apt to be crenated when the film has dried too slowly.

Pathologically, red corpuscles vary in size and shape, staining properties, and structure.

(1) **Variations in Size and Shape** (See Plate IX and Fig. 104).—The cells may be abnormally small (called *microcytes*, 5 μ or less in diameter); abnormally large (*macrocytes*, 10 to 12 μ); or extremely large (*megalocytes*, 12 to 20 μ).

Variation in shape is often very marked. Oval, pyriform, caudate, saddle-shaped, and club-shaped corpuscles are common (Fig. 85). They are called *poikilocytes*, and their presence is spoken of as poikilocytosis.

Red corpuscles which vary from the normal in size and shape are present in most symptomatic anemias, and in the severer grades are often very numerous. Irregularities are particularly conspicuous in leukemia and pernicious anemia, where, in some instances, a normal erythrocyte is the exception. In pernicious anemia there is a decided tendency to large size and oval forms, and megalocytes are rarely found in any other condition.

(2) **Variations in Staining Properties** (See Plate IX).—These include polychromatophilia, basophilic degeneration, and malarial stippling. With exception of polychromatophilia they are probably degenerative changes.

(*a*) *Polychromatophilia.*—Some of the corpuscles partially lose their normal affinity for acid stains, and take the basic stain to greater or less degree. Wright's stain gives such cells a faint bluish tinge when the condition is mild, and a rather deep blue when severe. Sometimes only part of a cell is affected. A few polychromatophilic corpuscles can be found in marked symptomatic anemias.
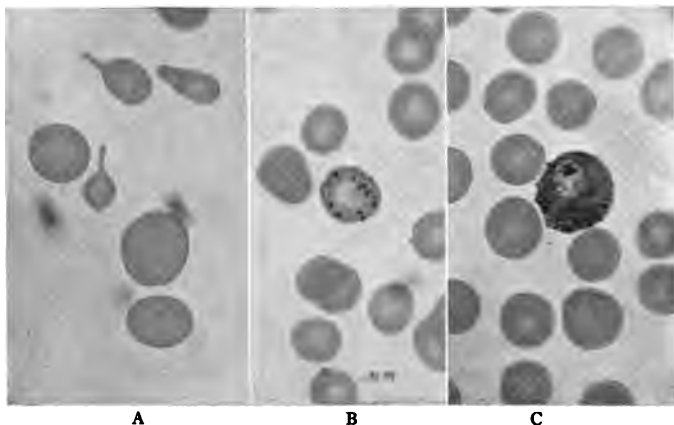
Fig. 85.—Abnormal red corpuscles: A, Poikilocytosis; B, basophilic granular degeneration; C, malarial stippling, the cell also containing a tertian parasite (× 1000) (courtesy of Dr. W. P. Harlow).

They occur most abundantly in malaria, leukemia, and pernicious anemia.

Polychromatophilia has been variously interpreted. It is thought by many to be evidence of youth in a cell, and hence to indicate an attempt at blood regeneration. There are probably several forms referable to different causes.

(*b*) *Basophilic Granular Degeneration* (*Degeneration of Grawitz*).—This is characterized by the presence, within

the corpuscle, of basophilic granules which vary in size from scarcely visible points to granules as large as those of basophilic leukocytes (Fig. 85). The number present in a red cell commonly varies in inverse ratio to their size. They stain deep blue with Wright's stain; not at all with Ehrlich's triple stain. The cell containing them may stain normally in other respects, or it may exhibit polychromatophilia.

Numerous cells showing this degeneration are commonly found in chronic lead-poisoning, of which they
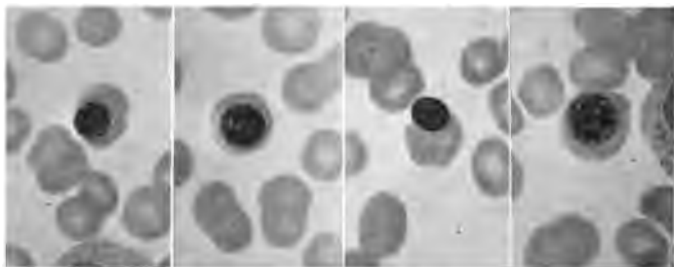
Fig. 86.—Normoblasts from cases of secondary anemia and leukemia (× 1000) (photographs by the author).

were at one time thought to be pathognomonic. Except in this disease, the degeneration indicates a serious blood condition. It occurs in well-marked cases of pernicious anemia and leukemia, and, much less commonly, in very severe symptomatic anemias.

(c) *Malarial Stippling.*—This term has been applied to the finely granular appearance often seen in red corpuscles, which harbor malarial parasites (Plates VI and VII and Fig. 85). It is commonly classed with the degeneration just described, but is probably distinct. Not all stains will show it. With Wright's stain it can be

brought out by staining longer and washing less than for the ordinary blood-stain. The minute granules stain reddish purple.

(3) **Variations in Structure.**—The most important is the presence of a nucleus (Plates VI and IX and Fig. 86). Nucleated red corpuscles, or *erythroblasts*, are classed according to their size: *microblasts*, 5 μ or less in diameter; *normoblasts*, 5 to 10 μ; and *megaloblasts*, above 10 μ. Microblasts and normoblasts contain one, rarely two, small round, sharply defined, deeply staining nuclei,

Fig. 87.—Megaloblasts from a case of pernicious anemia (× 1000) (courtesy of Dr. W. P. Harlow).

often located eccentrically. Occasionally the nucleus is irregular in shape, " clover-leaf " forms being not infrequent. The megaloblast (Fig. 87) is probably a distinct cell, not merely a larger size of the normoblast. Its nucleus is large, stains rather palely, has a delicate chromatin network, and often shows evidences of degeneration (karyorrhexis, etc.). In ordinary work, however, it is safer to base the distinction upon size than upon structure. Any nucleated red cell, but especially the megaloblast, may exhibit polychromatophilia.

Normally, erythroblasts are present only in the blood

of the fetus and of very young infants. In the adult, their presence in the circulating blood denotes an excessive demand upon the blood-forming organs to regenerate lost or destroyed red corpuscles. In response to this demand, immature and imperfectly formed cells are thrown into the circulation. Their number, therefore, is an indication of the extent to which the bone-marrow reacts rather than of the severity of the disease. Normoblasts occur in severe symptomatic anemia, leukemia, and pernicious anemia. They are most abundant in myelogenous leukemia. While always present in pernicious anemia, they are often difficult to find. Megaloblasts are found in pernicious anemia, and with extreme rarity in any other condition. They here almost invariably exceed the normoblasts in number, which is one of the distinctive features of the disease. Microblasts have much the same significance as normoblasts, but are less common.

Cabot's ring bodies are ring- or figure-of-8 shaped structures which have been observed in certain of the red cells in pernicious anemia, lead-poisoning, and lymphatic leukemia. They stain red with Wright's stain. Their nature is unknown.

2. The Leukocytes.—An estimation of the number or percentage of each variety of leukocyte in the blood is called a *differential count*. It probably yields more helpful information than any other single procedure in blood examinations.

The differential count is best made upon a film stained with Wright's, Harlow's, or Ehrlich's stain. Go carefully over the film with an oil-immersion lens, using a mechanical stage if available. Classify each leukocyte

seen, and calculate what percentage each variety is of the whole number classified. For accuracy, 500 to 1000 leukocytes must be classified; for approximate results, 300 are sufficient. Track of the count may be kept by placing a mark for each leukocyte in its appropriate column, ruled upon paper. Some workers divide a slidebox into compartments with slides, one for each variety of leukocyte, and drop a coffee-bean into the appropriate compartment when a cell is classified. When a convenient number of coffee-beans is used (any multiple of 100), the percentage calculation is extremely easy.

The actual number of each variety in a cubic millimeter of blood is easily calculated from these percentages, and the total leukocyte count. An increase in actual number is an *absolute increase;* an increase in percentage only, a *relative increase.* It is evident that an absolute increase of any variety may be accompanied by a relative decrease.

A record is generally kept of the number of nucleated red cells seen during a differential count of leukocytes.

The usual classification of leukocytes is based upon their size, their nuclei, and the staining properties of the granules which many of them contain. It is not altogether satisfactory, but is probably the best which our present knowledge permits.

The writer has found the table (Fig. 88, p. 232) very helpful in impressing this classification upon the student. It makes no attempt to indicate histogenetic relationships. The leukocytes of normal blood fall into two groups, each including three types. The cells in Group I contain single, round, oval or horseshoe-shaped nuclei, and have few or no granules in their cytoplasm.

The stippling of the cytoplasm shown in the diagram represents the finely granular appearance of protoplasm, not true granulation. The cells in Group II are polymorphonuclear and contain granules which are distinguished by their size and staining reactions. In its structure the chief abnormal leukocyte, the myelocyte, combines the

LEUKOCYTES

NORMAL

I { *MONONUCLEAR*
  { *NON-GRANULAR*

1-LYMPHOCYTE   20-30%

2-LARGE MONONUCLEAR }
                     } 4-8%
3 TRANSITIONAL   }

II { *POLYMORPHONUCLEAR*
   { *GRANULAR*

1- NEUTROPHILIC   60-75%

2 - EOSINOPHILIC   2-4%

3- BASOPHILIC   0.5%

ABNORMAL

NUCLEUS AS IN GROUP I

GRANULES AS IN GROUP II

MYELOCYTE

1 - NEUTROPHILIC

2 - EOSINOPHILIC

3 - BASOPHILIC
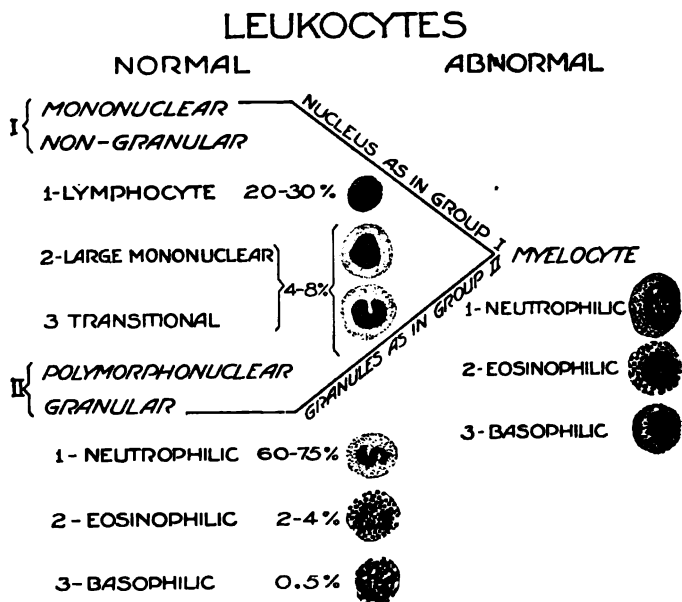
Fig. 88.—Outline of the classification of leukocytes.

two groups, being mononuclear like Group I, and granular like Group II.

(1) **Normal Varieties.**—(*a*) **Lymphocytes.**—These are small mononuclear cells without granules (Plates VI and X). They are about the size of a red corpuscle or slightly larger (6–10 μ), and consist of a single, sharply

Stained with Wright's stain. All drawn to same scale.

1, Normal red corpuscle for comparison; 2, normoblasts, one with lobulated nucleus; 3, megaloblast and microblast. The megaloblast shows a considerable degree of polychromatophilia; 4, blood-plaques, one lying upon a red corpuscle; 5, lymphocytes, large and small; 6, large mononuclear leukocyte; 7, transitional leukocyte; 8, polymorphonuclear neutrophilic leukocytes; 9, eosinophilic leukocytes, one ruptured; 10, basophilic leukocyte; 11, neutrophilic myelocyte. The granules are sometimes less numerous and less distinct than here shown; 12, eosinophilic myelocytes; 13, basophilic myelocyte; 14, "irritation" or "stimulation" form, with small vacuoles; 15, degenerated leukocytes: two polymorphonuclear neutrophiles, one ruptured, one swollen and vacuolated; and a "basket cell" composed of an irregular meshwork of nuclear material; 16, large mononuclear leukocyte containing pigment-granules; from a case of tertian malaria; 17, four stages in the asexual cycle of the tertian malarial parasite: the second and fourth were drawn from the same slide taken from a case of double tertian; 18, red corpuscle containing tertian parasite and showing malarial stippling; 19, estivo-autumnal malarial parasites: two small ring forms within the same red cell, and a crescent with remains of the red corpuscle in its concavity.

PLATE VI

defined, deeply staining nucleus, surrounded by a narrow rim of protoplasm. The nucleus is generally round, but is sometimes indented at one side. Wright's stain gives the nucleus a deep purple color and the cytoplasm a pale robin's-egg blue in typical cells. Larger forms of lymphocytes are frequently found, especially in the blood of children, and are difficult to distinguish from the large mononuclear leukocytes. It is possible that the larger
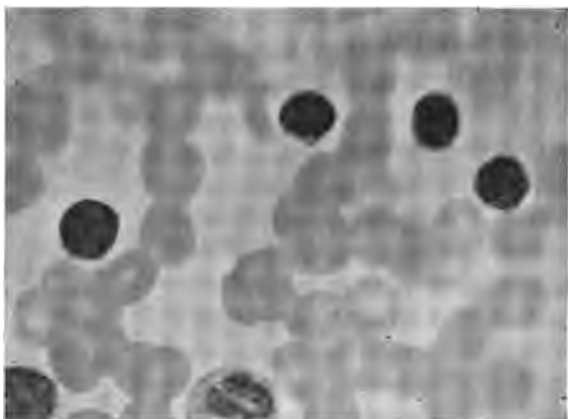
Fig. 89.—Lymphocytosis, case of pertussis (× 1000) (courtesy of Dr. W. P. Harlow).

forms are young lymphocytes, which become smaller as they grow older.

Lymphocytes are formed in the lymphoid tissues, including that of the bone-marrow. They constitute, normally, 20 to 30 per cent. of all leukocytes, or about 1000 to 3000 per c.mm. of blood. They are more abundant in the blood of children.

The percentage of lymphocytes is usually moderately increased in those conditions which give leukopenia,

especially typhoid fever, chlorosis, pernicious anemia, and many debilitated conditions. A marked increase, accompanied by an increase in the total leukocyte count, is seen in pertussis (Fig. 89) and lymphatic leukemia. In the latter the lymphocytes sometimes exceed 98 per cent. Exophthalmic goiter commonly gives a marked relative lymphocytosis, while simple goiter does not affect the lymphocytes.

(b) **Large Mononuclear Leukocytes** (Plate VI).— These cells are two or three times the diameter of the normal red corpuscle. Each contains a single round or oval nucleus, often located eccentrically. The zone of protoplasm surrounding the nucleus is relatively wide. With Wright's stain the nucleus is less deeply colored than that of the lymphocyte, while the cytoplasm is very pale blue or colorless, and sometimes contains a few reddish granules. The size of the cell, the width of the zone of cytoplasm, and the depth of color of the nucleus are the points to be considered in distinguishing between large mononuclears and lymphocytes. When large forms of the lymphocyte are present, the distinction is often difficult or impossible. It is then advisable to count the two cells together as lymphocytes. Indeed, they are regarded by some hematologists as identical.

Large mononuclear leukocytes probably originate in the bone-marrow or spleen. Some hold that they are developed from the endothelial cells of the blood-vessels. They constitute 2 to 4 per cent. of the total number of leukocytes: 100 to 400 per c.mm. of blood. An increase is unusual except in malaria, where it is quite constantly observed, and where many of the cells contain engulfed pigment.

(*c*) **Transitional Leukocytes** (Plate VI).—These are essentially large mononuclears with deeply indented or horseshoe-shaped nuclei. A few fine neutrophilic granules are sometimes present in their cytoplasm.

They are probably formed from the large mononuclears, and occur in the blood in about the same numbers.   The two cells are usually counted together, constituting 4 to 8 per cent. of the leukocytes.
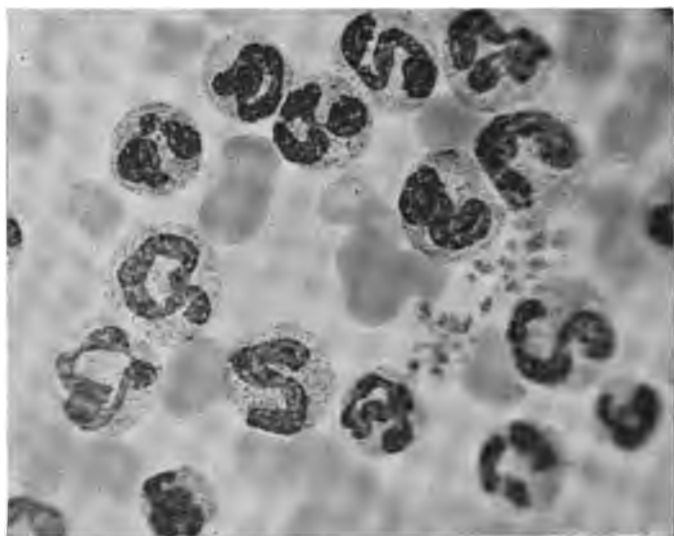
Fig. 90.—Marked polymorphonuclear neutrophilic leukocytosis (× 1000) (courtesy of Dr. W. P. Harlow).

(*d*) **Polymorphonuclear Neutrophilic Leukocytes** (Plate VI).—There is usually no difficulty in recognizing these cells.   Their average size is somewhat less than that of the large mononuclears.   The nucleus stains rather deeply, and is extremely irregular, often assuming shapes comparable to letters of the alphabet, E, Z, S, etc.

(Fig. 90). Frequently there appear to be several separate nuclei, hence the widely used name, "polynuclear leukocyte." Upon careful inspection, however, delicate nuclear bands connecting the parts can usually be seen. The cytoplasm is relatively abundant, and contains great numbers of very fine neutrophilic granules (Fig. 93). With Wright's stain the nucleus is bluish purple, and the granules reddish lilac.

Polymorphonuclear leukocytes are formed in the bone-marrow from neutrophilic myelocytes. They constitute 60 to 75 per cent. of all the leukocytes: 3000 to 7500 per c.mm. of blood. Increase in their number practically always produces an increase in the total leukocyte count, and has already been discussed under Polymorphonuclear Leukocytosis. The leukocytes of pus, *pus-corpuscles*, belong almost wholly to this variety.

A comparison of the percentage of polymorphonuclear cells with the total leukocyte count yields more information than a consideration of either alone. In a general way the percentage represents the severity of the infection, or, more correctly, the *degree of toxic absorption;* while the total count indicates the patient's *power of resistance*. With moderate infection and good resisting powers the leukocyte count and the percentage of poly-morphonuclears are increased proportionately. When the polymorphonuclear percentage is increased to a notably greater extent than is the total number of leukocytes, no matter how low the count, either very poor resistance or a very severe infection may be inferred.

Gibson has suggested the use of a chart to express this relationship graphically (Fig. 91). Its arrangement is purely arbitrary, but it may be found helpful in inter-

preting counts.   An ascending line from left to right
indicates an unfavorable prognosis in proportion as the
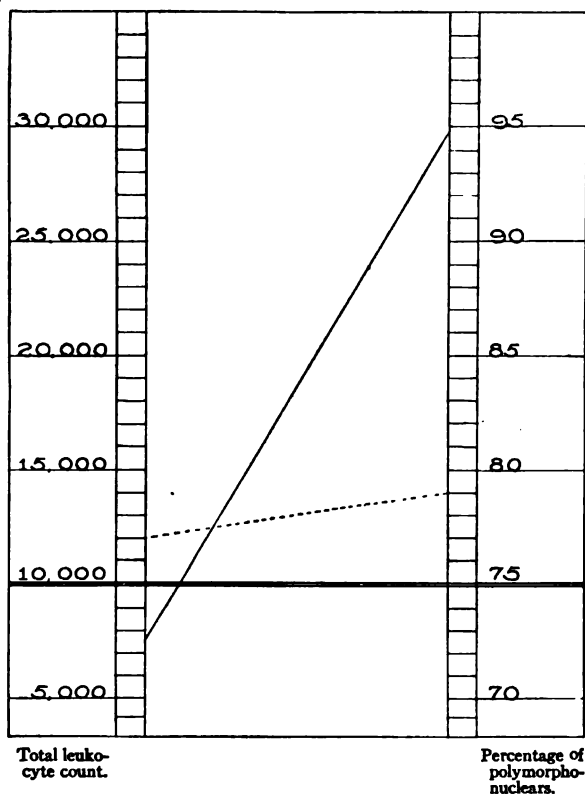
Fig. 91.—Gibson chart with blood-count in two cases of appendicitis: Dotted line representing a mild case with prompt recovery; the continuous line, a very virulent streptococcic case with poor resistance, peritonitis, and early death.

line approaches the vertical.   All fatal cases show a ris-
ing line.   A descending or horizontal line suggests a very
favorable prognosis.

It is a matter of observation that in the absence of acute infectious disease or of inflammation directly in the blood-stream (e. g., phlebitis, sigmoid sinusitis, septic endocarditis), a polymorphonuclear percentage of 85 or over points very strongly to gangrene or pus-formation somewhere in the body. On the other hand, excepting in children, where the percentage is normally low, pus is uncommon with less than 80 per cent. of polymorphonuclears.

Normally, the cytoplasm of leukocytes stains pale yellow with iodin. Under certain pathologic conditions the cytoplasm of many of the polymorphonuclears stains diffusely brown, or contains granules which stain reddish brown with iodin. This is called *iodophilia*. Extracellular iodin-staining granules, which are present normally, are more numerous in iodophilia.

This iodin reaction occurs in all purulent conditions except abscesses which are thoroughly walled off and purely tuberculous abscesses. It is of some value in diagnosis between serous effusions and purulent exudates, between catarrhal and suppurative processes in the appendix and Fallopian tube, etc. Its importance, however, as a diagnostic sign of suppuration has been much exaggerated, since it may occur in any general toxemia, such as pneumonia, influenza, malignant disease, and puerperal sepsis.

To demonstrate iodophilia, place the air-dried films in a stoppered bottle containing a few crystals of iodin until they become yellow. Mount in syrup of levulose and examine with an immersion objective.

Arneth classifies polymorphonuclear leukocytes into five groups, according to the number of lobes which the

nucleus shows. The percentage of cells in each group is fairly constant in health, but shows considerable variation in disease.

(e) **Eosinophilic Leukocytes, or "Eosinophiles"** (Plate VI).—The structure of these cells is similar to that of the polymorphonuclear neutrophiles, with the striking difference that, instead of fine neutrophilic granules, their cytoplasm contains coarse, round or oval granules having a strong affinity for acid stains. They are easily recognized by the size and color of the granules, which stain bright red with Wright's stain (Fig. 93). Their cytoplasm has generally a faint sky-blue tinge, and the nucleus stains somewhat less deeply than that of the polymorphonuclear neutrophile.

Eosinophiles are formed in the bone-marrow from eosinophilic myelocytes. Their normal number varies from 50 to 400 per c.mm. of blood, or 1 to 4 per cent. of the leukocytes. An increase is called *eosinophilia*, and is better determined by the actual number than by the percentage.

Slight eosinophilia is physiologic during menstruation. Marked eosinophilia is always pathologic. It occurs in a variety of conditions, the most important of which are: infection by animal parasites; bronchial asthma; myelogenous leukemia; scarlet fever, and many skin diseases.

(a) Eosinophilia may be a symptom of *infection by any of the worms*. It is fairly constant in trichinosis, uncinariasis, filariasis, and echinococcus disease. In this country an unexplained marked eosinophilia warrants examination of a portion of muscle for *Trichinella spiralis* (p. 363).

(b) *True bronchial asthma* commonly gives a marked eosinophilia during and following the paroxysms. This

is helpful in excluding asthma of other origin. Eosinophiles also appear in the sputum in large numbers.

(c) In *myelogenous leukemia* there is almost invariably an absolute increase of eosinophiles, although, owing to the great increase of other leukocytes, the percentage is usually diminished. Dwarf and giant forms are often numerous.

(d) *Scarlet fever* is frequently accompanied by eosinophilia, which may help to distinguish it from measles.
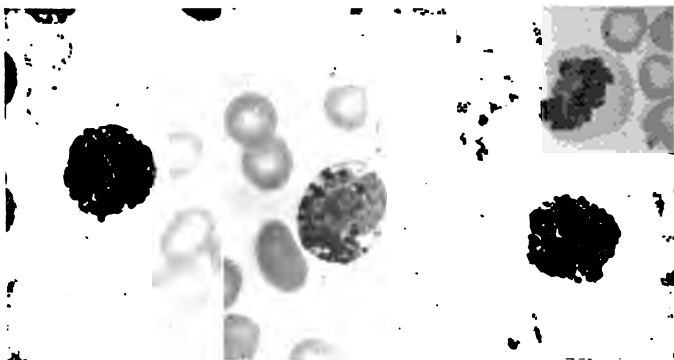
Fig. 92.—Basophilic leukocytes. At the right is also a normoblast undergoing mitosis (× 1000) (photographs by the author).

(e) Eosinophilia has been observed in a large number of *skin diseases*, notably pemphigus, prurigo, psoriasis, and urticaria. It probably depends less upon the variety of the disease than upon its extent.

(f) **Basophilic Leukocytes or " Mast-cells "** (Plate VI).—In general, these resemble polymorphonuclear neutrophiles except that the nucleus is less irregular and that the granules are larger and have a strong affinity for basic stains. They are easily recognized (Figs. 92 and 93). With Wright's stain the granules are deep purple, while

the nucleus is pale blue and is often nearly or quite hidden by the granules, so that its form is difficult to make out. These granules are not colored by Ehrlich's stain.

The nature of mast-cells is undetermined. They probably originate in the bone-marrow. They are least numerous of the leukocytes in normal blood, rarely exceeding 0.5 per cent., or 25 to 50 per c.mm. A notable increase is limited almost exclusively to myelogenous leukemia, where they are sometimes very numerous.
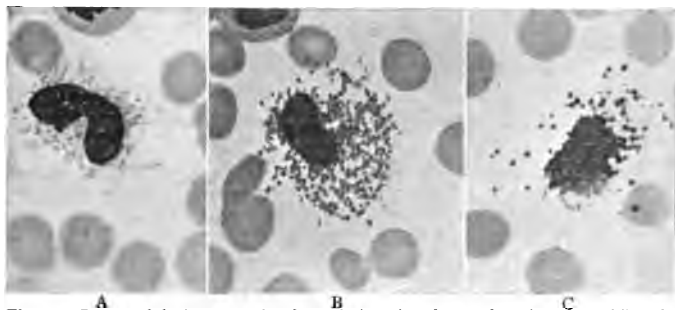
Fig. 93.—Ruptured leukocytes, showing relative size of granules: A, neutrophilic; B, eosinophilic; C, basophilic ($\times$ 1000) (photographs by the author).

(2) **Abnormal Varieties.**—(a) **Myelocytes** (Plate VI and Fig. 94).—These are large mononuclear cells whose cytoplasm is filled with granules. Typically, the nucleus occupies about one-half of the cell, and is round or oval. It is sometimes indented, with its convex side in contact with the periphery of the cell. It stains rather feebly. The average diameter of this cell (about 15.75 μ) is greater than that of any other leukocyte, but there is much variation in size among individual cells. Myelocytes are named according to the character of their granules— neutrophilic, eosinophilic, and basophilic myelocytes. These granules are identical with the corresponding

16

granules in the leukocytes just described. The occurrence of two kinds of granules in the same cell is rare.

Myelocytes are the bone-marrow cells from which the corresponding granular leukocytes are developed. Their presence in the blood in considerable numbers is diagnostic of myelogenous leukemia. The neutrophilic form is the least significant. A few of these may be present in very marked leukocytosis or any severe blood condition, as pernicious anemia. Eosinophilic myelocytes are found
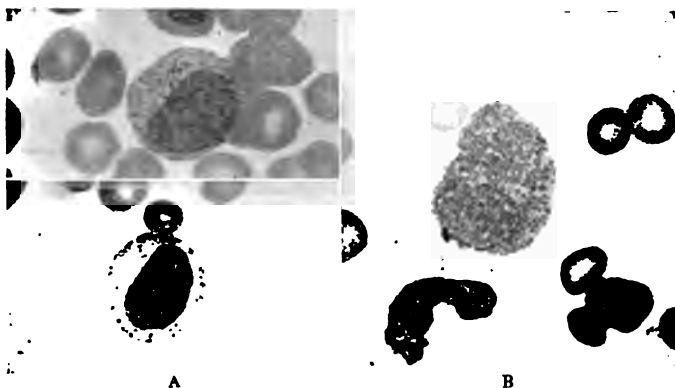
Fig. 94.—Myelocytes from blood of myelogenous leukemia: A, Neutrophilic; B, eosinophilic (× 1000) (photographs by the author).

only in myelogenous leukemia, where they are often very numerous. The basophilic variety is less common, and is confined to long-standing, severe myelogenous leukemia.

(b) **Atypic Forms.**—Leukocytes which do not fit in with the above classification are not infrequently met, especially in high-grade leukocytosis, pernicious anemia, and leukemia. The nature of most of them is not clear, and their number is usually so small that they may be

disregarded in making a differential count. Among them are:

(*a*) Border-line forms between polymorphonuclear neutrophiles and neutrophilic myelocytes.

(*b*) Small neutrophilic cells with a single round, deeply staining nucleus; they probably result from division of polymorphonuclear neutrophiles.

(*c*) " Irritation forms "—large non-granular mononuclear cells, whose cytoplasm stains fairly deep purple with Wright's stain, and intense brown with Ehrlich's:
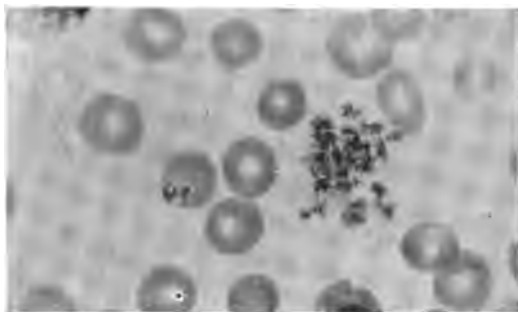
Fig. 95.—A cluster of blood-plaques and two plaques lying upon a red cell and simulating malarial parasites (× 1000) (photograph by the author).

they appear in the blood under the same conditions as myelocytes.

(*d*) Degenerated forms: vacuolated leukocytes, or merely palely or deeply staining homogeneous or reticulated masses of chromatin (the so-called " basket-cells," Plate VI).

3. Blood-plaques.—These are not colored by Ehrlich's stain nor by eosin and methylene-blue. With Wright's stain they appear as spheric or ovoid, reddish to violet, granular bodies, 2 to 4 μ in diameter. When well stained

a delicate hyaline peripheral zone can be distinguished. In ordinary blood-smears they are usually clumped in masses. A single platelet lying upon a red corpuscle may easily be mistaken for a malarial parasite (Plate VI and Fig. 95).

Blood-platelets are being much studied at present, but, aside from the facts mentioned under their enumeration (p. 213), little of clinical value has been learned. They have been variously regarded as very young red corpuscles (the "hematoblasts" of Hayem), as disintegration products of leukocytes, as remnants of extruded nuclei of erythrocytes, and as independent nucleated bodies. The most probable explanation of their origin seems to be that of J. H. Wright, who, from his recent studies, regards them as detached portions of the cytoplasm of certain giant-cells of the bone-marrow and spleen.

VIII. BLOOD PARASITES

A. BACTERIA

Bacteriologic study of the blood is useful in many conditions, but in general, the elaborate technic involved takes it out of the reach of the clinician. As applied to the diagnosis of typhoid fever, however, the technic of blood-cultures has been so simplified that it can be carried through by any one who is competent to do the simplest cultural work.

Typhoid bacilli can be detected in the blood in practically every case of typhoid fever in the first week of the disease; in about 80 to 85 per cent. of cases in the second week; and in decreasing percentages in the later weeks. The blood-culture, therefore, offers the most certain means

of early diagnosis. It is in a sense complementary to the Widal reaction, the former decreasing and the latter increasing in reliability as the disease progresses. The blood-culture gives best results before the Widal appears, as one would expect from the fact that the Widal test depends upon the presence of antibodies which destroy, or, at least injure, the bacilli. The two methods together will establish the diagnosis in practically every case at any stage. Bacilli disappear from the blood in convalescence and reappear in a relapse.

Technic of Blood-cultures in Typhoid Fever.—The blood may be obtained in one of two ways:

(*a*) With a spring-lancet make a deep puncture in the edge (not the side) of the lobe of the ear, as for a blood-count. Allow the blood to drop directly into a short culture-tube containing the bile medium. By gentle milking, 20 to 40 drops can usually be obtained. This simple method of obtaining blood is especially applicable during the first week of the disease when bacilli are abundant. Contamination with skin cocci is possible, but does not usually interfere when the bile medium is used.

(*b*) In the later weeks of the disease a larger quantity of blood is needed. Prepare the skin on the front of the elbow, as for a minor operation, or simply rub well with alcohol. Tie a bandage tightly around the upper arm, have the patient open and close the fist a few times, and when the veins are sufficiently distended insert a hypodermic needle attached to a syringe into any vein that is prominent. The needle should go through the skin about ¼ inch from the vein with the bevel at its tip uppermost, and should enter the vein from the side in a direction opposite to the blood-current (Fig. 96). Unless too small a needle is used, blood will begin to rise in the syringe as soon as the needle has entered the vein.

Suction is not necessary. When sufficient blood is obtained, the bandage is first removed, the needle is withdrawn, and the blood is allowed to run into a tube of culture-medium. It is usually easy to secure 5 to 10 c.c. of blood. The procedure causes the patient surprisingly little inconvenience, seldom more than does an ordinary hypodermic injection. There is rarely any difficulty in entering the vein except in children, and in adults when the arm is fat and the veins are small. If desired, one of the veins about the ankle can be used. Instead of a syringe one can use a large glass tube
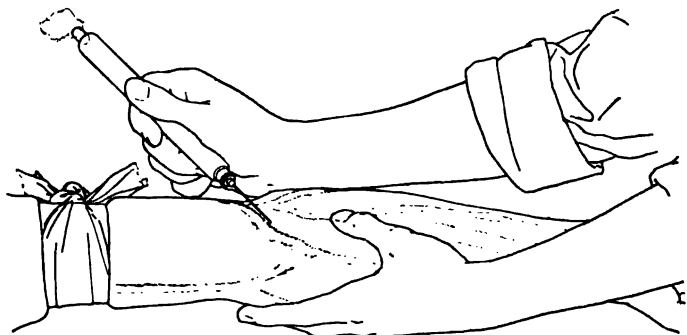
Fig. 96.—Method of obtaining blood for a blood-culture.

which has been drawn out at the ends and one end ground to fit a " slip-on " needle. Either a large hypodermic needle or a small antitoxin needle may be used. These little instruments (Fig. 96) can be made by any glass-blower at a cost of about fifty cents, and several of them can be kept on hand in test-tubes sterilized ready for use.

As special culture-medium, ox-bile is generally used. It favors the growth of the typhoid bacillus and retards the growth of other organisms. A good formula is given on p. 405.

As soon as convenient after the blood is added, place the tubes in the incubator. After about twelve hours examine

for motile bacilli. If none are found, transfer a few drops to tubes of bouillon or solidified blood-serum and incubate for twelve hours longer. If motile, Gram-negative bacilli are found; they are almost certainly typhoid bacilli. Further study is not necessary in practice, although desirable from a scientific point of view. The only bacilli which might cause confusion are the paratyphoid and colon bacilli, which can be distinguished by gas production in glucose media, indol production, and their effect upon litmus milk. The agglutination test for the identity of the bacillus is not available clinically, since freshly isolated bacilli do not agglutinate well.

B. Animal Parasites

Of the animal parasites which have been found in the blood, five are interesting clinically: the spirochæta of relapsing fever; trypanosomes; malarial parasites; filarial embryos; and the embryos of *Trichinella spiralis.*

1. Spirochæta recurrentis is described on p. 330.

2. Trypanosoma Gambiense.—Various trypanosomes are common in the blood of fishes, amphibians, birds, and mammals (Fig. 113). They live in the blood-plasma and do not attack the corpuscles. In some animals they are apparently harmless; in others they are an important cause of disease. They are discussed more fully on p. 333.

The trypanosome of human blood, *Trypanosoma gambiense* (Plate VII), is an actively motile, spindle-shaped organism, two or three times the diameter of a red corpuscle in length, with an undulating membrane which terminates at the anterior end in a long flagellum. It can be seen with medium power objectives in fresh blood, but is best studied with an oil-immersion lens in preparations stained as for the malarial parasite. Human trypano-

somiasis is common in Africa. As a rule, it is a very
chronic disease. "Sleeping sickness" is a late stage when
the organisms have invaded the cerebrospinal fluid.
Infection is carried by the tsetse fly, *Glossina palpalis.*

3. The Malarial Parasites.—These protozoa belong to
the Sporozoa (p. 338), order Hemosporidia, the mem-
bers of which are parasites in the blood of a great
variety of vertebrates. Three species, constituting the
genus *Plasmodium*, are associated with malarial fever in
man: *Plasmodium vivax*, *P. malariæ*, and *P. falciparum*,
the parasites respectively of the tertian, quartan, and
estivo-autumnal types of malaria. The life histories of
the three are so similar that they may well be described
together.

(1) **Life Histories.**—There are two cycles of develop-
ment: one, the *asexual*, in the blood of man; and the
other, the *sexual*, in the intestinal tract of a particular
genus of mosquito, *Anopheles.*

(*a*) *Asexual Cycle.*—The young organism enters the
blood through the bite of the mosquito. It makes its way
into a red corpuscle, where it appears as a small, pale
"hyaline" body. This body exhibits ameboid movement
and increases in size. Soon, dark-brown granules derived
from the hemoglobin of the corpuscle make their appear-
ance within it. When it has reached its full size—filling
and distending the corpuscle in the case of the tertian
parasite, smaller in the others—the pigment granules
gather at the center or at one side; the organism divides
into a number of small hyaline bodies, the spores or
merozoites; and the red corpuscle bursts, setting spores
and pigment free in the blood-plasma. This is called
segmentation. It coincides with, and by liberation of

PLATE VII

Trypanosoma gambiense (slide presented by Professor F. G. Novy).
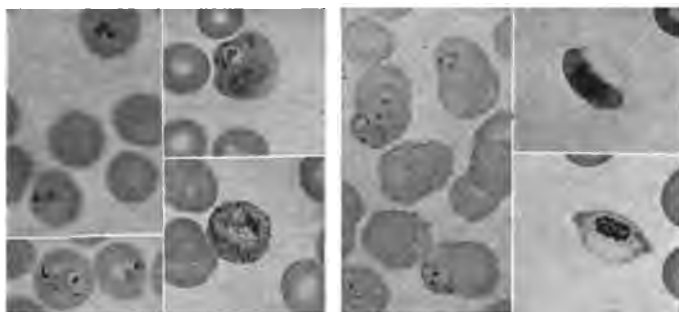
Tertian malarial parasites, one red cell showing malarial stippling.

Estivo-autumnal malarial parasites, small ring forms and crescents.

Spirochæta novyi.

Animal parasites of the blood; × 1000 (photographs by the author).

toxins causes, the paroxysm of the disease. A considerable number of the spores are destroyed by leukocytes or other agencies; the remainder enter other corpuscles and repeat the cycle. Many of the pigment granules are taken up by leukocytes. In estivo-autumnal fever segmentation occurs in the internal organs and the segmenting and larger pigmented forms are not seen in the peripheral blood.

The asexual cycle of the tertian organism occupies forty-eight hours; of the quartan, seventy-two hours; of the estivo-autumnal, an indefinite time—usually twenty-four to forty-eight hours.

The parasites are thus present in the blood in great groups, all the individuals of which reach maturity and segment at approximately the same time. This explains the regular recurrence of the paroxysms at intervals corresponding to the time occupied by the asexual cycle of the parasite. Not infrequently there is multiple infection, one group reaching maturity while the others are still young; but the presence of two groups which segment upon the same day is extremely rare. Fevers of longer intervals—six, eight, ten days—are probably due to the ability of the body, sometimes of itself, sometimes by aid of quinin, to resist the parasites, so that numbers sufficient to cause a paroxysm do not accumulate in the blood until after several repetitions of the asexual cycle. In estivo-autumnal fever the regular grouping, while usually present at first, is soon lost, thus causing "irregular malaria."

(b) *Sexual Cycle.*—Besides the ameboid individuals which pass through the asexual cycle, there are present with them in the blood many individuals with sexual

properties. These are called *gametes*. They do not undergo segmentation, but grow to adult size and remain inactive in the blood until taken up by a mosquito. Many of them are apparently extracellular, but stained preparations usually show them to be surrounded by the remains of a corpuscle. In tertian and quartan malaria they cannot easily be distinguished from the asexual individuals until a variable time after the blood leaves the body, when the male gamete sends out one or more flagella. In estivo-autumnal malaria the gametes take distinctive ovoid and crescentic forms, and are not difficult to recognize. They are very resistant to quinin and often persist in the blood long after the ameboid forms have been destroyed, but are probably incapable of continuing the disease until they have passed through the cycle in the mosquito.

When a malarious person is bitten by a mosquito, the gametes are taken with the blood into its stomach. Here a flagellum from the male unites with the female, which soon thereafter becomes encysted in the wall of the intestine. After a time it ruptures, liberating many minute rods, or sporozoites, which have formed within it. These migrate to the salivary glands, and are carried into the blood of the person whom the mosquito bites. Here they enter red corpuscles as young malarial parasites, and the majority pass through the asexual cycle just described.

The sexual cycle can take place only within the body of one genus of mosquito, *Anopheles*. Absence of this mosquito from certain districts explains the absence of malaria. It is distinguished from our common house-mosquito, *Culex*, by the relative lengths of proboscis and palpi (Fig. 97), which can be seen with a hand-lens, by

its attitude when resting, and by its dappled wing (Fig. 98). Anopheles is strictly nocturnal in its habits; it usually flies low, and rarely travels more than a few hundred yards from its breeding-place, although it may be carried by winds. These facts explain certain peculiarities in malarial infection; thus, infection occurs practically only at night; it is most common near stagnant water, especially upon the side toward which the prevailing winds blow; and the danger is greater when per-
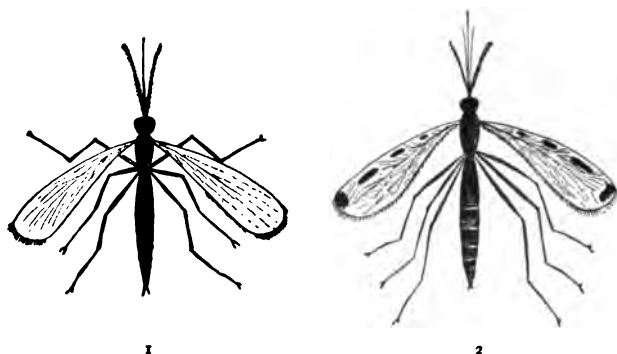
Fig. 97.—Mosquitoes—*Culex* (1) and *Anopheles* (2) (Bergey).

sons sleep upon or near the ground than in upper stories of buildings. The insects frequently hibernate in warmed houses, and may bite during the winter. A mosquito becomes dangerous in eight to fourteen days after it bites a malarious person, and remains so throughout its life.

(2) **Detection.**—Search for the malarial parasite may be made in either fresh blood or stained films. If possible, the blood should be obtained a few hours before the chill —never during it nor within a few hours afterward, since

at that time (in single infections) only the very young, unpigmented forms are present, and these are the most difficult to find and recognize. Sometimes many parasites are found in a microscopic field; sometimes, especi-
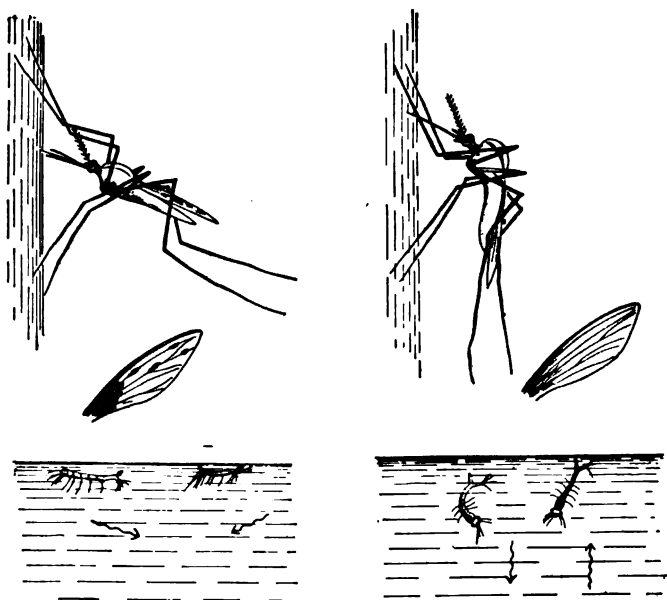
Fig. 98.—Showing, on the left, *Anopheles* in resting position, its dappled wing, and the position of its larvæ in water; on the right, *Culex* in resting position, its plain wing, and the position of its larvæ in water. The arrows indicate the directions taken by the larvæ when the water is disturbed (Abbott).

ally in estivo-autumnal infection, owing to accumulation in internal organs, careful search is required to find any, despite very severe symptoms. Quinin causes them rapidly to disappear from the peripheral blood, and few or none may be found after its administration. In the

absence of organisms, the presence of pigment granules within leukocytes—polymorphonuclears and large mono-nuclears—may be taken as presumptive evidence of malaria. Pigmented leukocytes (Plate VI) are most numerous after a paroxysm.

(*a*) *In Fresh Unstained Blood* (Plate VIII).—Obtain a small drop of blood from the finger or lobe of the ear. Touch the center of a cover-glass to the top of the drop and quickly place it, blood side down, upon a slide. If the slide and cover be perfectly clean and the drop not too large, the blood will spread out so as to present only one layer of corpuscles. Search with an oil-immersion objective, using very subdued light.

The young organisms appear as small, round, ring-like or irregular, colorless bodies within red corpuscles. The light spots caused by crenation and other changes in the corpuscles are frequently mistaken for them, but are generally more refractive or have more sharply defined edges. The older forms are larger colorless bodies con-taining granules of brown pigment. In the case of the tertian parasite, these granules have active vibratory motion, which renders them conspicuous; and as the parasite itself is very pale, one may see only a large pale corpuscle in which fine pigment granules are dancing. Segmenting organisms, when typic, appear as rosets, often compared to daisies, the petals of which represent the segments, while the central brown portion represents the pigment. Tertian segmenting forms are less fre-quently typic than quartan. Flagellated forms are not seen until ten to twenty minutes after the blood has left the vessels. As Cabot suggests, one should, while search-ing, keep a sharp lookout for unusually large or pale cor-

puscles, and for anything which is brown or black or in
motion.

(b) *In Stained Films* (Plates VI and VII).—Recogni-
tion of the parasite, especially the young forms, is much
easier in films stained by Wright's or some similar stain
than in fresh blood.   When very scarce, they may some-
times be found, although their structure is not well shown
by the method of Ruge. This consists in spreading a very
thick layer of blood, drying, placing for a few minutes in
a fluid containing 5 per cent. formalin and 1 per cent.
acetic acid, which removes the hemoglobin and fixes the
smear, rinsing, drying, and finally staining.   Carbol-
thionin is very useful for this purpose.   If Wright's
stain be used in this method, it is recommended that the
preparation be subsequently stained for a half-minute
with borax-methylene-blue (borax, 5; methylene-blue, 2;
water, 100).

In films which are properly stained with Wright's fluid
the young organisms are small, round, ring-like or irreg-
ular, sky-blue bodies, each with a very small, sharply de-
fined, reddish-purple chromatin mass.   Many structures
—deposits of stain, dirt, blood-plaques lying upon red
cells (Fig. 95), etc.—may simulate them, but should not de-
ceive one who looks carefully for both the blue cytoplasm
and the reddish-purple chromatin.   A plaque upon a red
corpuscle is surrounded by a colorless zone rather than by
a distinct blue body.   Young estivo-autumnal parasites
commonly take a " ring " form (the chromatin mass rep-
resenting the jewel), which is infrequently assumed by
the other varieties.   The older tertian and quartan or-
ganisms show larger sky-blue bodies with more reticular
chromatin, and contain brown granules of pigment, which,

Various forms of malarial parasites (unstained) (Thayer and Hewetson).

1 to 10, inclusive, Tertian organisms; 11 to 17, inclusive, quartan organisms; 18 to 27, inclusive, estivo-autumnal organisms; 1, young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, extracellular pigmented form; 10, flagellate form; 11, young hyaline form; 12, 13, pigmented forms; 14, fully developed pigmented form; 15, 16, segmenting forms; 17, flagellate form; 18, 19, 20, ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

PLATE VIII

however, is less evident than in the living parasite. The chromatin is often scattered through the cytoplasm or apparently outside of it, and is sometimes difficult to see clearly. Typical "segmenters" present a ring of rounded segments or spores, each with a small, dot-like chromatin mass. With the tertian parasite, the segments more frequently form an irregular cluster. The pigment is collected near the center or scattered among the segments. In estivo-autumnal fever usually only the small "ring bodies" and the crescentic and ovoid gametes are seen
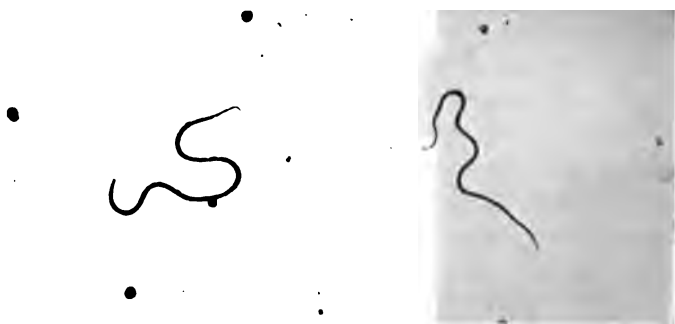
Fig. 99.—Filarial embryos in blood. Stained. Red corpuscles decolorized; a few leukocytes remain (× 200) (photographs by the author).

in the blood. The gametes are easily recognized. Their length is somewhat greater than the diameter of a red corpuscle. Their chromatin is usually centrally placed, and they contain more or less coarse pigment. The remains of the red cell often form a narrow rim around them or fill the concavity of the crescent.

While the parasites are more easily found in stained preparations, the varieties are more easily differentiated in fresh blood. The chief distinguishing points are included in the table on page 256.

VARIETIES OF THE MALARIAL ORGANISM

TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL.
Asexual cycle, forty-eight hours.	Seventy-two hours.	Usually twenty-four to forty-eight hours.
Substance pale, transparent, comparable to hyaline tube-cast.	Highly refractive, comparable to waxy tube-cast.	Highly refractive.
Outline indistinct.	Distinct.	Distinct.
Ameboid motion active.	Sluggish.	Active.
Mature asexual form large; fills and often distends corpuscle.	Smaller.	Young forms, only, in peripheral blood.
Pigment - granules fine, brown, scattered throughout. Very active dancing motion.	Much coarser, darker in color, peripherally arranged. Motion slight.	Very few, minute, inactive. Distinctly pigmented forms seldom seen.
Segmenting body rarely assumes typical "daisy" form. 15 to 20 segments.	Usually typical "daisy." 6 to 12 segments.	Not seen in peripheral blood.
Gametes resemble asexual forms.	Same as tertian.	Appear in blood as distinctive ovoids and crescents.
Red corpuscles pale and swollen.	Generally darker than normal.	Dark, often bronzed.

4. Filarial Embryos.—A description of the filariæ whose embryos appear in the blood will be found on p. 356.

The embryos can be seen in stained preparations, (Fig. 99), but are best found in fresh unstained blood. A rather large drop is taken upon a slide, covered, and examined with a low power. The embryo can be located by the commotion which its active motion produces

among the corpuscles. This motion consists almost wholly in apparently purposeless lashing and coiling movements, and continues for many hours.

5. Embryos of Trichinella Spiralis.—The worm and its life-history are described on page 363. It has recently been shown that diagnosis of trichiniasis can frequently be made by detection of the embryos in the blood during their migration to the muscles. Of eleven such examinations which have been reported within the past two and a half years, six were positive. The earliest time at which the embryos were found was the sixth day after the onset of symptoms; the latest, the twenty-second day.

The method is very simple. One to 10 c.c. of blood are obtained from the ear or a vein, as described on page 245, and mixed with ten times its volume of 3 per cent. acetic acid. The mixture is centrifugalized, and large drops of the sediment are placed on slides, covered, and searched with a low-power objective. The embryos are not difficult to recognize. They are about $125\,\mu$ long and $6\,\mu$ broad.

IX. SERUM REACTIONS

1. Agglutination.—In the blood-serum of persons suffering from certain infectious diseases there exist soluble bodies, called agglutinins, which have the property of rendering non-motile and clumping the specific microorganism of the disease, and have little or no influence upon other bacteria. This " agglutination " takes place even when the blood is greatly diluted. Undiluted normal blood can agglutinate most bacteria, but loses this power when diluted to any considerable degree. These

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facts are taken advantage of in the diagnosis of several diseases.

When applied to the diagnosis of typhoid fever, the phenomenon is known as the *Widal reaction*. As yet, it is the only agglutination reaction which has any practical value for the practitioner.

Either blood-serum or the whole blood may be used. Serum is the better. To obtain it, it is convenient to use little vials, such as can be made by breaking off the lower
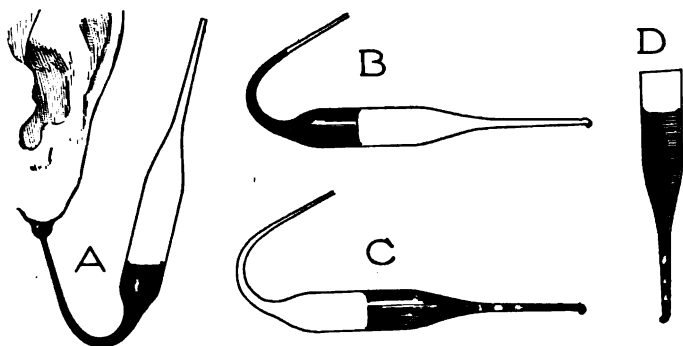
Fig. 100.—Method of obtaining blood in a Wright capsule: A, Filling the capsule; B, the bulb has been warmed and the capillary end sealed in a flame; C, cooling of the capsule has drawn the blood to the sealed end; D, the serum has separated, and the top of the capsule has been broken off.

half-inch of the tubes which have contained peptonizing powder. They must, of course, be well cleaned. One of these is filled to a depth of about $\frac{1}{4}$ inch from a puncture in the finger or the ear, and is set aside for a few hours. When the clot has separated, it is picked out with a needle, leaving the serum. It is, however, more satisfactory to obtain the blood in a Wright capsule (Fig. 100). This capsule is easily made from a piece of glass-tubing as indicated in Fig. 161.

One drop of the serum is then added to nine drops of normal salt solution, making a dilution of 1 : 10. Distilled water may be used for dilution, but is more liable to cause error. The dilution can be more accurately made in the leukocyte pipet of the Thoma-Zeiss instrument. When the whole blood is used, it can be secured in this pipet and at once diluted with the salt solution. When it must be transported a considerable distance, dried blood is most convenient. A large drop is allowed to dry upon a clean slide or unglazed paper. It will keep for months without losing its agglutinating power. When ready to make the test, the dried stain is dissolved in ten drops of normal salt solution, care being taken that the drops are about the same size as the original drop of blood.

The reaction can be detected either microscopically or macroscopically:

Microscopic Method.—(1) The blood or serum having been obtained and diluted 1 : 10 as just described, mix it with a bouillon culture of the typhoid bacillus to any desired dilution. One drop of each makes a blood-dilution of 1 : 20, etc. The culture should be between eighteen and twenty-four hours old, and the bacilli must be actively motile. A stock agar culture should be kept at room temperature, and bouillon tubes inoculated the day before the examination is to be made. Agar cultures can be purchased from dealers in biologic products. They must be renewed monthly.

Instead of the bouillon culture, McFarland recommends the use of a suspension made by removing some of the growth from the surface of a fresh agar culture and mixing it well with a little sterile water. It is then necessary to examine the suspension microscopically to make sure that there are no natural clumps.

(2) Place a few drops of the mixture of blood and culture upon a perfectly clean slide and apply a cover-glass. The cover may be ringed with vaselin to prevent evaporation, but this is not usually necessary.

(3) Examine at intervals with a high dry lens—a 4 mm. will answer very well. The light must be very subdued. At first the bacilli should be actively moving about. If the blood be from a case of typhoid, they will gradually lose their motion and gather together in clumps (Fig. 101). The clumps should be large, and the few bacilli remaining isolated should

Fig. 101.—Showing clumping of typhoid bacilli in the Widal reaction. At one point a crenated red blood-corpuscle is seen (Wright and Brown).

be motionless. Pseudoreactions, in which there are a few small clumps of bacilli whose motion is not entirely lost, together with many freely moving bacilli scattered throughout the field, should not mislead. As a control, a drop of the culture should always be examined before making the test.

Normal blood may produce clumping if time enough be allowed. The diagnostic value of a positive reaction is, therefore, impaired unless clumping takes place within a limited time. With dilution of 1 : 40 the time limit should not exceed

forty-five minutes; with 1 : 80, one and one-half hours. Tests based upon lower dilution than 1 : 40 are probably not reliable.

Macroscopic Method.—The principle is the same as that of the microscopic method. Clumping of the bacilli causes a flocculent precipitate, which can be seen with the naked eye. A dead culture gives the same results as a living one. This method is as reliable as the microscopic and is more convenient for the practitioner, although it requires more time.

Dead cultures, together with apparatus for diluting the blood, are put up at slight cost by various firms, under the names of typhoid diagnosticum, typhoid agglutometer, etc. Full directions accompany these outfits and need not be repeated here.

Recently, Bass and Watkins have described a modification of the macroscopic method (using very concentrated suspensions of the bacilli) by which the test can be applied at the bedside. Clumping occurs within two minutes. The apparatus has been put upon the market by Parke, Davis & Co.

The Widal reaction is positive in over 95 per cent. of all cases of typhoid fever. It may, rarely, be positive in other conditions, owing, sometimes at least, to faulty technic. It seldom appears before the fifth or sixth day; usually during the second week, but sometimes not until convalescence. It is, therefore, of less value in early diagnosis than is the blood-culture (p. 244). When it once appears it remains during the whole course of the disease, and frequently persists for years.

2. Opsonins.—That phagocytosis plays an important part in the body's resistance to bacterial invasion has long been recognized. According to Metchnikoff, this

property of leukocytes resides entirely within themselves, depending upon their own vital activity. The studies of Wright and Douglas, upon the contrary, indicate that the leukocytes are impotent in themselves, and can ingest bacteria only in the presence of certain substances which exist in the blood-plasma. These substances have been named *opsonins*. Their nature is undetermined. · They probably act by uniting with the bacteria, thus preparing them for ingestion by the leukocytes; but they do not cause death of the bacteria, nor produce any appreciable morphologic change. They appear to be more or less specific, a separate opsonin being necessary for phagocytosis of each species of bacteria. There are, moreover, opsonins for other formed elements—red blood-corpuscles, for example. It has been shown that the quantity of opsonins in the blood can be greatly increased by inoculation with dead bacteria.

To measure the amount of any particular opsonin in the blood Wright has devised a method which involves many ingenious and delicate technical procedures. Much skill, such as is attained only after considerable training in laboratory technic, is requisite, and there are many sources of error. It is, therefore, beyond the province of this work to recount the method in detail. In a general way it consists in: (*a*) Preparing a mixture of equal parts of the patient's blood-serum, an emulsion of the specific micro-organism, and a suspension of washed leukocytes; (*b*) preparing a similar mixture, using serum of a normal person; (*c*) incubating both mixtures for a definite length of time; and (*d*) making smears from each, staining, and examining with an oil-immersion objective. The number of bacteria which have been taken up by a definite

number of leukocytes is counted, and the average number of bacteria per leukocyte is calculated; this gives the "phagocytic index." The phagocytic index of the blood under investigation, divided by that of the normal blood, gives the *opsonic index* of the former, the opsonic index of the normal blood being taken as 1. Simon regards the percentage of leukocytes which have ingested bacteria as a more accurate measurement of the amount of opsonins than the number of bacteria ingested, because the bacteria are apt to adhere and be taken in in clumps.

Because of its simplicity the clinical laboratory worker will prefer some modification of the Leishman method, which uses the patient's own leukocytes. It is, perhaps, as accurate as the original method of Wright, although variations in the leukocyte count have been shown to affect the result. Two pipets like those shown in Fig. 164 are used.

(1) Make a suspension of the specific organism by mixing a loopful of a young agar culture with 1 c.c. of a solution containing 1 per cent. sodium citrate and 0.85 per cent. sodium chlorid. Thoroughly break up all clumps by sucking the fluid in and forcing it out of one of the capillary pipets held vertically against the bottom of the watch-glass.

(2) Puncture the patient's ear, wipe off the first drop of blood, and from the second draw blood into the other pipet to the grease pencil mark, let in a bubble of air, and draw in the same amount of bacterial suspension.

(3) Mix upon a slide by drawing in and forcing out of the pipet.

(4) Draw the mixture high up in the pipet, seal the tip in the flame, and place in the incubator for fifteen minutes.

(5) Repeat steps 2, 3, and 4 with the blood of a normal person.

(6) After incubation, break off the tip of the pipet, mix the blood-bacteria mixture, and spread films on slides.

(7) Stain with Wright's or Harlow's blood-stain.

(8) With an oil-immersion lens count the bacteria which have been taken in by 100 leukocytes, and calculate the average number per leukocyte. Divide the average for the patient by the average for the normal person. This gives the opsonic index. If in the patient's blood there was an average of 4 bacteria per leukocyte, and in the normal blood 5 bacteria per leukocyte, the opsonic index would be ⅘ or 0.8.

Wright and his followers regarded the opsonic index as an index of the power of the body to combat bacterial invasion. They claimed very great practical importance for it as an aid to diagnosis and as a guide to treatment by the vaccine method. This method of treatment consists in increasing the amount of protective substances in the blood by injections of normal salt suspensions of dead bacteria of the same species as that which has caused and is maintaining the morbid process, these bacterial suspensions being called "vaccines." Vaccine Therapy (Chapter IX) has taken a permanent place among our methods of treatment of bacterial infections, particularly of those which are strictly local, but the opsonic index is now little used either as a measure of resisting power or as an aid to diagnosis and guide to treatment.

3. Wassermann Reaction.[1]—The Wassermann test for syphilis, like the Widal test for typhoid fever, de-

[1] By Clough T. Burnett, Professor of Bacteriology, University of Colorado.

pends upon the detection in the patient's blood-serum of specific antibodies, *agglutinins* in the case of typhoid, *immune bodies* or *amboceptors* in the case of syphilis. These antibodies have been produced by the tissues in response to the entrance of the invading organism. If they are present, it is assumed that the patient has or has had syphilis. The Wassermann test is, however, much more complicated than the Widal test, and can be properly performed only by a trained laboratory worker. It is the aim here to explain only the general principles of the method, together with its clinical significance. For a proper understanding of the test the principles of bacteriolysis and hemolysis must first be presented.

Bacteriolysis and Hemolysis.—In 1894 Pfeiffer, working with guinea-pigs immunized to cholera, found that when living cholera germs were introduced into the peritoneal cavity of an immune animal they lost their motility within a few minutes, and very shortly were seen to disintegrate and go into complete solution. This has been known as Pfeiffer's phenomenon, or *bacteriolysis*. It was later demonstrated that this reaction could take place outside the animal body if the bacteria were mixed in the test-tube with the blood-serum or peritoneal fluid of a cholera immune animal. Subsequent researches showed that while an old or heated immune serum failed to cause this solution of the bacteria, upon the addition of a normal fresh serum this property returned. This addition of a normal serum to a serum which has lost its solvent action is called reactivation of the serum. These changes may best be demonstrated by the following chart:

BACTERIOLYSIS.

Immune serum, fresh	+ bacteria		= *solution.*
Normal " "	+ "		= no solution.
Immune " heated	+ "		= no solution.
Immune " "	+ normal serum + bacteria		= *solution.*

From the chart it is clear that there are two substances concerned in bacteriolysis, one of which is found in any fresh serum, but is easily destroyed, and is called the *complement.* The other substance is found only in the immune serum, is relatively stable, and is known as the *immune body* or *amboceptor*.

In hemolysis we find an analogy to bacteriolysis. Let a rabbit be immunized to sheep's blood-corpuscles. Now, if washed sheep's blood-corpuscles be subjected to the action of fresh serum from this rabbit, a speedy solution of the red cells ensues. If this serum is allowed to stand for several days, or is heated one-half hour to 56° C., it will completely lose its solvent power. Now, the addition of a fresh normal serum, even of another species, will reactivate the heated or old serum. The following chart will indicate these reactions:

HEMOLYSIS.

Rabbit serum, immune	+ corpuscles (sheep's)		= *solution.*
Rabbit " " heated	+ " "		= no solution.
Normal "	+ " "		= no solution.
Rabbit " " heated	+ normal serum + corpuscles (sheep's)		
= *solution.*			

In hemolysis, as in bacteriolysis, besides the antigen (substance giving rise to amboceptors or antibodies) there are two substances. One of these is specific, *i. e.*, only found in immune serum, and reacting only with the substance used in producing the immune serum. This sub-

stance is relatively stable, and is known as the amboceptor. The other substance is found in any serum, is absolutely non-specific, is easily destroyed, and is called the complement. In neither case will the amboceptor nor the complement acting alone cause a solution of the antigen.

There are three substances necessary to bacteriolysis and hemolysis. To produce bacteriolysis there must be the specific antigen (as cholera vibrio in Pfeiffer's phenomenon), the immune serum containing the amboceptor, and a complement. These three substances comprise the bacteriolytic system. Likewise, in hemolysis there is the red blood-cell, the amboceptor, and a complement, which comprise the hemolytic system.

It will be noted that there is one factor common to both systems, viz., the complement. It will be evident that if we place in a test-tube a complete bacteriolytic system, with just enough complement to cause solution of the bacteria, and place this for a sufficient time in the optimum temperature for bacteriolysis, and then add two elements of the hemolytic system (amboceptor and blood-cells), no hemolysis will ensue, because all of the complement was used by the bacteriolytic system.

Bordet and Gengou in 1901 showed that it was possible to utilize this fact in the diagnosis of certain bacterial infections. For instance, the heated serum of a suspected typhoid case plus typhoid bacilli is added to a serum containing complement (fresh guinea-pig serum) and incubated one hour. If this suspected serum contains typhoid amboceptors, there will have been a combination effected between the three elements of the bacteriolytic system, so that there will be no free complement left. If no amboceptor is present, all of

the complement will remain unattached. Now, in order to show whether this complement has been fixed or deviated, two elements of a hemolytic system are added—namely, amboceptor and red corpuscles, and if the complement is fixed, no hemolysis can ensue.

This is easily understood from a study of the swinging pendulum diagram, in which the complement is represented in the pendulum.
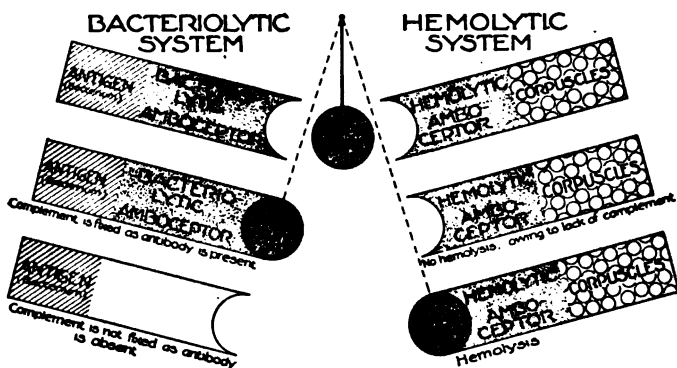
Fig. 102.—Pendulum diagram illustrating hemolysis.[1]

This principle of "complement deviation" having been utilized in the diagnosis of infectious diseases, it occurred to Wassermann in 1906 to apply it to the diagnosis of syphilis. The antigen first used was the extract of a fetal syphilitic liver, but subsequent work has shown that the same reaction may be obtained with normal liver or spleen tissue, or with certain lipoid substances, and in this sense is not a true antigen-antibody reaction. The antibodies in the syphilitic blood, however, are specific. These are analogous to the bacteriolytic amboceptor of the pendulum diagram.

[1] Not original, but unable to place credit where due.

Technic of the Wassermann Test.—The following reagents are necessary:

Antigen.—Extract of fetal syphilitic or normal liver, diluted 1 : 10.

Antibodies (Analogous to Bacteriolytic Antibodies).—The serum or spinal fluid of the suspected patient. As controls, the serum of a syphilitic known to contain antibodies and the serum of a normal person known not to contain antibodies.

Complement.—Fresh guinea-pig serum. Other fresh normal sera may be used. This is diluted 1 : 10.

Hemolytic Amboceptor.—The serum of a rabbit which has been immunized to sheep's red blood-corpuscles. This serum is inactivated before use by heating to 56° C. one-half hour, and diluted 1 : 1000 before using.

Corpuscle Suspension.—Sheep's blood is defibrinated, washed three times with normal salt solution, and then diluted with normal salt solution to make a 5 per cent. suspension.

In using these various reagents it is necessary to know that they are potent and of the proper strength, that is, to establish the titre of the reagent. This being determined, we are now ready for the Wassermann test, as carried out in the table on page 270.

In the luetic control tube there will occur a combination between the antibodies in the serum and the antigen, which together will cause a fixation of the complement, so that when later the two elements of the hemolytic system are added, no hemolysis will occur. This inhibition of hemolysis indicates a positive syphilitic reaction.

Control tube No. 3 is used to show that there is nothing in a normal serum which can effect this combination and deviation. Tube No. 4 shows that the patient's serum alone is not anticomplementary. Tube No. 5 shows that the hemolytic system is effective. Tube No. 6 shows that the

WASSERMANN REACTION

TUBE I	TUBE II	TUBE III	TUBE IV.	TUBE V.	TUBE VI	TUBE VII.
SUSPECTED.	LUETIC CONTROL	NORMAL CONTROL	CONTROL	CONTROL	CONTROL	CONTROL
c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Patient's serum, 0.2	Luetic serum, 0.2	Normal serum, 0.2	Patient's serum, 0.2	....	....	....
Complement,[1] 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	....
Antigen,[2] 0.4	Antigen, 0.4	Antigen, 0.4	....	....	Antigen, 0.4	Antigen, 0.4
Salt solution, 2.0	Salt solution, 2.0	Salt solution, 2.0	Salt solution, 2.4	Salt solution, 2.6	Salt solution, 2.2	Salt solution, 2.6
Incubate one hour at 37° C.						
Amboceptor,[3] 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	....
Corpuscles,[4] 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0
Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 1.0
Incubate two hours at 37° C.						
Make observations first; then set aside in cold for eighteen to twenty-four hours and take final reading.						
No hemolysis if syphilitic.	No hemolysis.	Hemolysis.	Hemolysis.	Hemolysis.	Hemolysis.	No hemolysis.

[1] The complement is a 1:10 dilution of guinea-pig serum.
[2] This is the antigen diluted 1:10.
[3] The serum of rabbit which has been immunized to sheep's blood-corpuscles is diluted 1:1000 and constitutes the amboceptor.
[4] A 5 per cent. suspension of washed lamb's corpuscles.

antigen alone is not anticomplementary. Tube No. 7 is introduced to show that hemolysis will not occur in the absence of the complement.

Modifications.—Certain modifications of this test have been suggested, chief of which is the **Noguchi test.** This differs from the Wassermann mainly in that an anti-human hemolytic system is used instead of an anti-sheep, because, according to the author, there is an appreciable error in the Wassermann in that there is present " in human serum a variable amount of natural antisheep amboceptor " capable of so changing the results that with sera containing only a small amount of syphilitic antibody the result will be negative.

The following reagents are used:

1. Antihuman hemolytic amboceptor prepared by repeated injections of a rabbit with washed human blood-corpuscles.

2. Complement. Fresh guinea-pig serum.

3. Antigen. Organ extracts or a solution of lecithin.

4. 1 per cent. suspension of human blood-corpuscles.

5. The suspected serum.

6. A known syphilitic serum.

7. A serum known not to contain syphilitic antibodies.

With these reagents the procedure is very little different from that outlined for the Wassermann test.

Noguchi has further simplified it for the small laboratory by drying the amboceptor serum on slips of filter-paper, which can be kept a considerable time. The same procedure can be carried out with the antigen. While at first similar complement slips were prepared, it is now known that fresh complement is indispensable.

Value of Wassermann Test.—The reaction is positive in 95 to 98 per cent. of all cases with syphilitic manifestations. In the late cases only a very slight inhibition of hemolysis may be noted. This has given rise to considerable difficulty in the interpretation of results. Kaplan states that the report should read "negative" or "positive," with no report of the degree of inhibition.

Butler obtains the following results:

	No. of cases.	Per cent. positive.
Controls, non-syphilitic............	53	o
Primary syphilitic.................	10	100
Secondary syphilitic..............	36	95
Tertiary syphiltic.................	31	94
Latent cases.....................	16	56
Parasyphilis and visceral syphilis.....	55	76
Total cases...............	201	

Kaplan, in a study of diseases of the nervous system, obtained the following results: In 249 cases of quiescent tabes the Wassermann reaction was positive in 44 per cent.; in 57 cases of active tabes, 88 per cent., and in 64 cases of general paresis, 88 per cent.

By the Noguchi method about 7 per cent. of non-syphilitic sera will cause inhibition of hemolysis, while with the Wassermann, in about 9 per cent. of known syphilitic sera, hemolysis will occur. For this reason in doubtful cases it is well to apply both methods.

It is probable that a positive reaction almost always means active syphilis even without manifestations, but it is not absolutely specific for syphilis, for the reaction has been obtained in leprosy. Kaplan states that "old leprosy cases present a much more definitely positive reaction than cases of old syphilis." Many workers believe that a positive reaction in a late case may only

indicate that the patient has once had syphilis. Against this view stands the fact that in other infectious diseases antibodies diminish or entirely disappear a few months after the active infection, and that in latent cases the reaction may disappear under treatment. On the other hand, there are certain cases which are considered clinically as cured, and have remained so for years, that will continue to give the positive reaction in spite of any treatment.

In the application of the test to the diagnosis of diseases of the nervous system and viscera one should always bear in mind the possibility of a dual pathologic process, and a positive test should not be allowed to entirely overshadow the clinical findings.

Effect of Treatment.—The positive reaction frequently disappears after a short course of treatment with mercury. This may be permanent, or, after a variable length of time, the reaction may return. Some cases thoroughly treated persist in giving a positive reaction. In hereditary syphilis it is often impossible to get rid of the reaction. Because the reaction may return, it is always safer to make several tests before deciding that further treatment is not indicated. This suggests that while a positive reaction may be accepted as an indication of syphilis, a negative reaction obtained after treatment may not exclude syphilis.

Following treatment with salvarsan ("606"), Noguchi, in a total of 102 cases, finds that the positive reaction becomes negative in 33.7 per cent.

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X. TESTS FOR RECOGNITION OF BLOOD

1. Guaiac Test.—The technic of this test has been given (p. 125). It may be applied directly to a suspected fluid or, better, to the ethereal extract. Add a few cubic centimeters of glacial acetic acid to about 10 c.c. of the fluid; shake thoroughly with an equal volume of ether; decant, and apply the test to the ether. In case of dried stains upon cloth, wood, etc., dissolve the stain in distilled water and test the water, or press a piece of moist blotting-paper against the stain, and touch the paper with drops of the guaiac and the turpentine successively.

2. Teichmann's Test.—This depends upon the production of characteristic crystals of *hemin*. It is a sensi-

Fig. 103.—Teichmann's hemin crystals (Jakob).

tive test and, when positive, is absolute proof of the presence of blood. A number of substances—lime, fine sand, iron rust—interfere with production of the crystals; hence negative results are not always conclusive. Dissolve the suspected stain in a few drops of normal

salt solution upon a slide. If a liquid is to be tested, evaporate some of it upon a slide and dissolve the residue in a few drops of the salt solution. Let dry, apply a cover-glass, and run glacial acetic acid underneath it. Heat *very gently* until bubbles begin to form, replacing the acid as it evaporates. Allow to cool slowly. When cool, replace the acid with water, and examine for hemin crystals with 16 mm. and 4 mm. objectives. The crystals are dark-brown rhombic plates, lying singly or in crosses, and easily recognized (Fig. 103). Failure to obtain them may be due to too great heat or too rapid cooling. If not obtained at first let the slide stand in a warm place, as upon a hot-water radiator, for an hour.

XI. SPECIAL BLOOD PATHOLOGY

The more conspicuous characteristics of the blood in various diseases have been mentioned in previous sections. Although the great majority of blood changes are secondary, there are a few blood conditions in which the changes are so prominent, or the etiology so obscure, that they are commonly regarded as blood diseases. These will receive brief consideration here.

A. ANEMIA

This is a deficiency of hemoglobin, or red corpuscles, or both. It is either primary or secondary. The distinction is based chiefly upon etiology, although each type presents a more or less distinctive blood-picture. Secondary anemia is that which is symptomatic of some other pathologic condition. Primary anemia is that which progresses without apparent cause.

1. Secondary Anemia.—The more important conditions which produce secondary or symptomatic anemia are:

(*a*) *Poor nutrition*, which usually accompanies unsanitary conditions, poor and insufficient food, etc.

(*b*) *Acute infectious diseases*, especially rheumatism and typhoid fever. The anemia is more conspicuous during convalescence.

(*c*) *Chronic Infectious Diseases.*—Tuberculosis, malaria, syphilis, leprosy.

(*d*) *Chronic exhausting diseases*, as heart disease, chronic nephritis, cirrhosis of the liver, and gastro-intestinal diseases, especially when associated with atrophy of gastric and duodenal glands. The last may give an extreme anemia, indistinguishable from pernicious anemia.

(*e*) *Chronic poisoning*, as from lead, arsenic, and phosphorus.

(*f*) *Hemorrhage.*—Either repeated small hemorrhages, as from gastric cancer and ulcer, uterine fibroids, etc., or a single large one.

(*g*) *Malignant Tumors.*—These affect the blood partly through repeated small hemorrhages, partly through toxic products, and partly through interference with nutrition.

(*h*) *Animal Parasites.*—Some cause no appreciable change in the blood; others, like the hookworm and *Dibothriocephalus latus*, may produce a very severe anemia, almost identical with pernicious anemia. Anemia in these cases is probably due both to toxins and to abstraction of blood.

The blood-picture varies with the grade of anemia.

Diminution of hemoglobin is the most characteristic feature. In mild cases it is slight, and is the only blood change to be noted. In very severe cases hemoglobin may fall to 15 per cent. Red corpuscles are diminished in all but very mild cases, while in the severest cases the red corpuscle count is sometimes below 2,000,000. The color-index is usually decreased.

Although the number of leukocytes bears no relation to the anemia, leukocytosis is common, being due to the same cause.

Stained films show no changes in very mild cases. In moderate cases variations in size and shape of the red cells and polychromatophilia occur. Very severe cases show the same changes to greater degree, with addition of basophilic degeneration and the presence of normoblasts in small or moderate numbers. Megaloblasts in very small numbers have been encountered in extremely severe cases. They are especially abundant and may even predominate over the normoblasts in dibothriocephalus infection. Blood-plaques are usually increased.

2. Primary Anemia.—The commonly described varieties of primary anemia are pernicious anemia and chlorosis, but splenic anemia may also be mentioned under this head.

(1) **Progressive Pernicious Anemia.**—It is frequently impossible to diagnose this disease from the blood examination alone. Severe secondary anemia sometimes gives an identical picture. Remissions, in which the blood approaches the normal, are common. All the clinical data must, therefore, be considered.

Hemoglobin and red corpuscles are always greatly

diminished. In none of Cabot's 139 cases did the count exceed 2,500,000, the average being about 1,200,000. In more than two-thirds of the cases hemoglobin was reduced to less extent than the red corpuscles; the color-index was, therefore, high. A low color-index probably indicates a mild type of the disease.
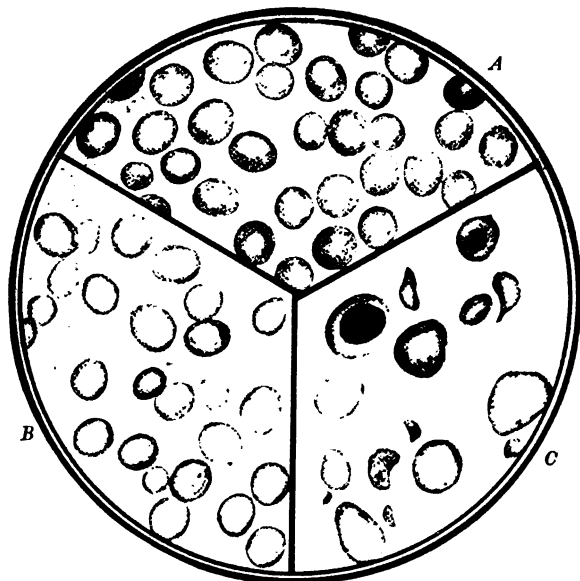
Fig. 104.—*A*, Normal blood; *B*, chlorosis; *C*, pernicious anemia. The plate shows the sharp contrast between cells rich in hemoglobin and the pale cells of chlorosis, and also the poikilocytes and marked variations in size noted in pernicious anemia. A normoblast and megaloblast also appear. Stained smears (from Greene's "Medical Diagnosis").

The leukocyte count may be normal, but is commonly diminished to about 3000. The decrease affects chiefly the polymorphonuclear cells, so that the lymphocytes are relatively increased. In some cases a decided absolute increase of lymphocytes occurs. Polymorphonuclear leukocytosis, when present, is due to some complication.

Fig. 1.—Preparation from an advanced case of progressive pernicious anemia from unknown cause: *a*, Megaloblasts or gigantoblasts; the protoplasm shows marked polychromasia; *b*, stained granules in erythrocytes with normally stained protoplasm; *c* and *d*, polychromatophilic degeneration; *e*, megalocytes; *f*, normocytes.

Fig. 2.—Preparation from the same case taken some time later while the patient was subjectively and objectively in perfect health: *a*, Punctate erythrocytes with normal and anemic degenerated protoplasm; *b*, polynuclear leukocyte; *c*, normal red blood-corpuscles; *d*, somewhat enlarged erythrocytes.

Fig. 3.—Series of cells from a case of severe progressive pernicious anemia of unknown etiology; preparation made two days antemortem: *a*, Nucleated red blood-corpuscles characterized as normoblasts by the intense staining of the nuclei; *a'* and *a"*, karyokinetic figures in erythrocytes; the protoplasm finely punctate; *b*, beginning karyolysis in a megaloblast; *c*, erythroblasts with coarse granulation of the protoplasm; *d*, nuclear remains (?) and fine granulation of the protoplasm; *e* and *f*, finely punctate red blood-corpuscles; *g*, megalocyte with two blue nuclei; nuclear remains (?) in the polychrome protoplasm.

(Nothnagel-Lazarus.)

PLATE IX

Fig. 1.

Fig. 2.
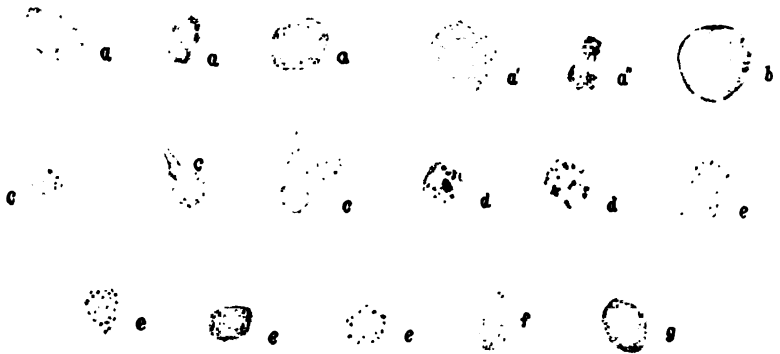
Fig. 3.

The red corpuscles show marked variation in size and shape (Plate IX and Fig. 104). There is a decided tendency to large oval forms, and despite the abundance of microcytes, the average size of the corpuscles is generally strikingly increased. Polychromatophilia and basophilic degeneration are common. Nucleated red cells are always present, although *in many instances careful search is required to find them.* In the great majority of cases megaloblasts exceed normoblasts in number. This ratio constitutes one of the most important points in diagnosis, since it is practically unknown in other diseases. Blood-plaques are diminished.

The rare and rapidly fatal anemia which has been described under the name of **aplastic anemia** is probably a variety of pernicious anemia. Absence of any attempt at blood regeneration explains the marked difference in the blood-picture. Red corpuscles and hemoglobin are rapidly diminished to an extreme degree. The color-index is normal or low. The leukocyte count is normal or low, with relative increase of lymphocytes. Stained smears show only slight variations in size, shape, and staining properties of the red cells. There are no megaloblasts and few or no normoblasts.

(2) **Chlorosis.**—The clinical symptoms furnish the most important data for diagnosis. The blood resembles that of secondary anemia in many respects.

The most conspicuous feature is a decided decrease of hemoglobin (down to 30 or 40 per cent. in marked cases), accompanied by a slight decrease in number of red corpuscles. The color-index is thus almost invariably low, the average being about 0.5.

As in pernicious anemia, the leukocytes are normal or

decreased in number, with a relative increase of lymphocytes.

In contrast to pernicious anemia (and in some degree also to secondary anemia) the red cells are of nearly uniform size, are uniformly pale (Fig. 104), and their average diameter is somewhat less than normal. Changes in size, shape, and staining reactions occur only in severe cases. Erythroblasts are rarely present. The number of plaques is generally decreased.

(3) **Splenic Anemia.**—This is an obscure form of anemia associated with great enlargement of the spleen. It is probably a distinct entity. There is decided decrease of hemoglobin and red corpuscles, with moderate leukopenia and relative lymphocytosis. Osler's 15 cases averaged 47 per cent. hemoglobin and 3,336,357 red cells. Stained films show notable irregularities in size, shape, and staining properties only in advanced cases. Erythroblasts are uncommon.

B. LEUKEMIA

Except in rare instances, diagnosis is easily made from the blood alone. Two types of the disease are commonly distinguished: the *myelogenous* and the *lymphatic*. Atypical and intermediate forms are not uncommon. Pseudoleukemia, because of its clinical similarity to lymphatic leukemia, is generally described along with leukemia.

1. **Myelogenous Leukemia** (Plate X).—This is usually a chronic disease, although acute cases have been described.

Hemoglobin and red corpuscles show decided decrease. The color-index is moderately low.

PLATE X

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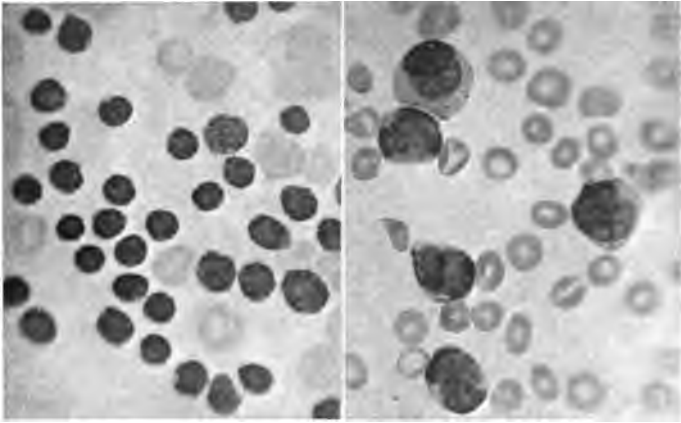
Fig. 1.—Blood in lymphatic leukemia; × 700. On the left, chronic form of the disease; on the right, acute form (courtesy of Dr. W. P. Harlow).
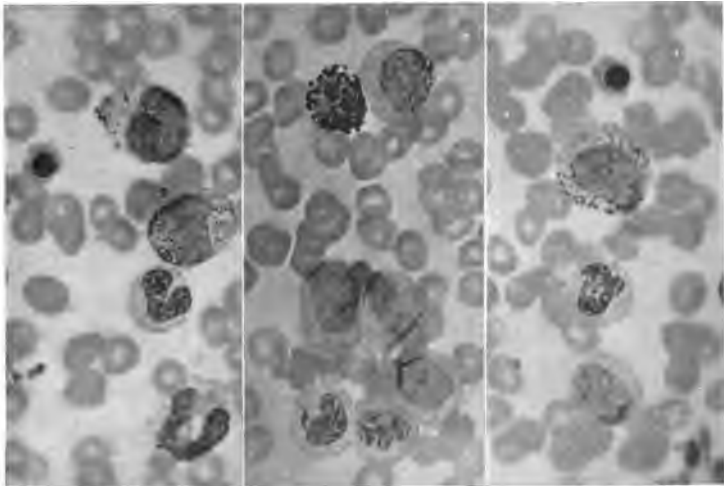
Fig. 2.—Blood in splenomyelogenous leukemia. Wright's stain. × 700 (photographs by the author).

Most striking is the immense increase in number of leukocytes. The count in ordinary cases varies between 100,000 and 300,000. Counts over 1,000,000 have been met. During remissions, the leukocyte count may fall to normal.

While these enormous leukocyte counts are equaled in no other disease, and approached only in lymphatic leukemia and extremely high-grade leukocytosis, the diagnosis, particularly during remissions, depends more upon qualitative than quantitative changes. Although all varieties are increased, the characteristic and conspicuous cell is the myelocyte. This cell never appears in normal blood; extremely rarely in leukocytosis; and never abundantly in lymphatic leukemia. In myelogenous leukemia myelocytes usually constitute more than 20 per cent. of all leukocytes. Da Costa's lowest case gave 7 per cent. The neutrophilic form is generally much more abundant than the eosinophilic. Both show considerable variations in size. Very constant also is a marked absolute, and often a relative, increase of eosinophiles and basophiles. Polymorphonuclear neutrophiles and lymphocytes are relatively decreased.

The red cells show the changes characteristic of a severe secondary anemia, except that nucleated reds are commonly abundant; in fact, no other disease gives so many. They are chiefly of the normoblastic type. Megaloblasts are uncommon. Blood-plaques are generally increased.

2. Lymphatic Leukemia (Plate X).—This form may be either acute or chronic. There is marked loss of hemoglobin and red corpuscles. The color-index is usually moderately low.

The leukocyte count is high, but lower than in the myelogenous type. Counts of 100,000 are about the average, but in many cases are much lower. This high count is referable almost wholly to increase of lymphocytes. They generally exceed 90 per cent. of the total number. In chronic cases they are chiefly of the small variety; in acute cases, of the large form. Myelocytes are rare.

The red corpuscles show the changes usual in severe secondary anemia. Erythroblasts are seldom abundant. Blood-plaques are decreased.

3. Pseudoleukemia (Hodgkin's disease) resembles lymphatic leukemia in that there is marked and progressive enlargement of the lymph-nodes. There is, however, no distinctive blood-picture. The changes in hemoglobin and red cells resemble those of a moderate symptomatic anemia, with rather low color-index. The leukocytes are commonly normal in number and relative proportions.

4. Anæmia Infantum Pseudoleukæmica.—Under this name von Jaksch described a rare disease of infancy, the proper classification of which is uncertain. There is enlargement of liver and spleen, and sometimes of lymph-nodes, together with the following blood changes: grave anemia with deformed and degenerated red cells and many erythroblasts of both normoblastic and megaloblastic types; great increase in number of leukocytes (20,000 to 100,000) and great variations in size, shape, and staining of leukocytes, with many atypic forms, and a few myelocytes.

The table on the following page contrasts the distinctive blood-changes in the more common conditions.

DIFFERENTIAL DIAGNOSIS OF BLOOD DISEASES

	Hemoglobin.	Red Cor-puscle Count.	Color-index.	Leucocyte Count.	Red Corpuscles. (Stained Films.)	Leucocytes. (Stained Films.)
Secondary anemia.	Diminished according to degree of diminished anemia.	Normal in mild cases; slightly diminished.	Normal or arbitrarily affect-ed; leukocy-tosis com-mon.	Not necess-arily affect-ed; variations in staining re-actions and normoblasts in severe cases.	Variations in size and shape in moderate cases; increase of polynuclears.	Normal proportions or increase of polynuclears.
Pernicious anemia.	Diminished	Greatly diminished.	High.	Normal or diminished.	Marked variations in size, shape, and staining reac-tions. Average size in-creased. Tendency to large oval forms. Erythroblasts always present; megalo-blasts exceed normoblasts.	Lymphocytes relatively, sometimes absolutely, in-creased.
Chlorosis.	Greatly di-minished.	Slightly di-minished.	Low.	Normal or diminished.	Nearly uniform size and shape; average size de-creased; pale centers. Erythroblasts very rare.	Lymphocytes apt to be relatively increased.
Myelogen-ous leuke-mia.	Decidedly diminished.	Decidedly diminished.	Usually slightly di-minished.	Extremely high.	Similar to secondary ane-mia, except normoblasts generally very numerous.	Large numbers of mye-locytes (average, 20 per cent.). Absolute increase of eosinophiles and baso-philes. Relative decrease of polynuclears and lymph-ocytes.
Lymphatic leukemia.	Markedly diminished.	Markedly diminished.	Usually slightly di-minished.	Very high.	Similar to secondary ane-mia. Erythroblasts not numerous.	Lymphocytes exceed 90 per cent. Other varieties relatively decreased.

CHAPTER IV

THE STOMACH

LABORATORY methods may be applied to the diagnosis of stomach disorders in: I. Examination of the gastric contents removed with the stomach-tube. II. Certain other examinations which give information as to the condition of the stomach.

I. EXAMINATION OF THE GASTRIC CONTENTS

Stomach digestion consists mainly in the action of pepsin upon proteins in the presence of hydrochloric acid and in the curdling of milk by rennin. The fat-splitting ferment, lipase, of the gastric juice has very little activity under normal conditions of acidity.

Pepsin and rennin are secreted by the gastric glands as zymogens—pepsinogen and renninogen respectively —which are converted into pepsin and rennin by hydrochloric acid. Hydrochloric acid is secreted by certain cells of the fundus glands. It at once combines loosely with the proteins of the food, forming acid-metaprotein, the first step in protein digestion. Hydrochloric acid, which is thus loosely combined with proteins, is called "combined" hydrochloric acid. The acid which is secreted after the proteins present have all been converted into acid-metaprotein remains as "free" hydrochloric acid, and, together with pepsin, continues the process of digestion.

At the height of digestion the stomach-contents consist essentially of: (1) Water; (2) free hydrochloric acid; (3) combined hydrochloric acid; (4) pepsin; (5) rennin; (6) mineral salts, chiefly acid phosphates, of no clinical importance; (7) particles of undigested and partly digested food; (8) various products of digestion in solution. In pathologic conditions there may be present, in addition, various microscopic structures and certain organic acids, of which lactic acid is most important.

A **routine examination** is conveniently carried out in the following order:

(1) Give the patient a test-meal upon an empty stomach, washing the stomach previously if necessary.

(2) At the height of digestion, usually in one hour, remove the contents of the stomach with a stomach-tube.

(3) Measure and examine macroscopically.

(4) Filter. A suction filter is desirable, and may be necessary when much mucus is present.

(5) During filtration, examine microscopically and make qualitative tests for—(a) free acids; (b) free hydrochloric acid; (c) lactic acid.

(6) When sufficient filtrate is obtained, make quantitative estimations of—(a) total acidity; (b) free hydrochloric acid; (c) combined hydrochloric acid (if necessary).

(7) Make whatever additional tests seem desirable, as for blood, pepsin, or rennin.

A.   Obtaining the Contents

Gastric juice is secreted continuously, but quantities sufficiently large for examination are not usually obtainable from the fasting stomach.   In clinical work, therefore, it is desirable to stimulate secretion with food—

which is the natural and most efficient stimulus—before attempting to collect the gastric fluid. Different foods stimulate secretion to different degrees, hence for the sake of uniform results certain standard "test-meals" have been adopted. Those mentioned here give practically the same results.

1. Test-meals.—It is customary to give the test-meal in the morning, since the stomach is most apt to be empty at that time. If it be suspected that the stomach will not be empty, it should be washed out with water the evening before.

(1) **Ewald's test-breakfast** consists of a roll (or two slices of bread), without butter, and two small cups (300 to 400 c.c.) of water, or weak tea, without cream or sugar. It should be well masticated. The contents of the stomach are to be removed one hour afterward, counting from the beginning, not the end of the meal. This test-meal has long been used for routine examinations. Its disadvantage is that it introduces, with the bread, a variable amount of lactic acid and numerous yeast-cells. This source of error may be eliminated by substituting a shredded whole-wheat biscuit for the roll. The shredded wheat test-meal is now widely used and is probably the most satisfactory for general purposes.

(2) **Boas' test-breakfast** consists of a tablespoonful of rolled oats in a quart of water, boiled to one pint, with a pinch of salt added. It should be withdrawn in forty-five minutes to one hour. This meal does not contain lactic acid, and is usually given when the detection of lactic acid is important, as in suspected gastric cancer. The stomach should always be washed with water the evening previous.

2. Withdrawal of the Contents.—The Boas stomach-tube, with bulb, is probably the most satisfactory form. It should be of rather large caliber, and have an opening in the tip and one or two in the side near the tip.   When not in use it should be kept in a vessel of borax solution, and should be well washed in hot water both before and after using.

It is important confidently to assure the patient that introduction of the tube cannot possibly harm him; and that, if he can control the spasm of his throat, he will experience very little choking sensation.   When patients are very nervous it is well to spray the throat with cocain solution.

The tube should be dipped in warm water just before using: the use of glycerin or other lubricant is undesirable.   With the patient seated upon a chair, his clothing protected by towels or a large apron, and his head tilted forward, the tip of the tube, held as one would a pen, is introduced far back into the pharynx.   He is then urged to swallow, and the tube is pushed boldly into the esophagus until the ring upon it reaches the incisor teeth, thus indicating that the tip is in the stomach.   If, now, the patient cough or strain as if at stool, the contents of the stomach will usually be forced out through the tube. Should it fail, the fluid can generally be pumped out by alternate compression of the tube and the bulb.   If unsuccessful at first, the attempts should be repeated with the tube pushed a little further in, or withdrawn a few inches, since the distance to the stomach is not the same in all cases.   The tube may become clogged with pieces of food, in which case it must be withdrawn, cleaned, and reintroduced.   If, after all efforts, no fluid

is obtained, another test-meal should be given and withdrawn in forty-five minutes.

As the tube is removed, it should be pinched between the fingers so as to save any fluid that may be in it.

The stomach-tube must be used with great care, or not at all, in cases of gastric ulcer, aneurysm, uncompensated heart disease, and marked arteriosclerosis. Except in gastric ulcer, the danger lies in the retching produced, and the tube can safely be used if the patient takes it easily.

B. Physical Examination

Under normal conditions 30 to 50 c.c. of fluid can be obtained one hour after administering Ewald's breakfast. More than 60 c.c. points to motor insufficiency; less than 20 c.c., to too rapid emptying of the stomach, or else to incomplete removal. Upon standing, it separates into two layers, the lower consisting of particles of food, the upper of an almost clear, faintly yellow fluid. The extent to which digestion has taken place can be roughly judged from the appearance of the food-particles.

The *reaction* is frankly acid in health and in nearly all pathologic conditions. It may be neutral or slightly alkaline in some cases of gastric cancer and marked chronic gastritis, or when contaminated by a considerable amount of saliva.

A small amount of *mucus* is present normally. Large amounts, when the gastric contents are obtained with the tube and not vomited, point to chronic gastritis. Mucus is recognized from its characteristic slimy appearance when the fluid is poured from one vessel into another. It is more frequently seen in stomach washings than in the fluid removed after a test-meal.

A trace of *bile* may be present as a result of excessive straining while the tube is in the stomach. Large amounts are very rarely found, and generally point to obstruction in the duodenum. Bile produces a yellowish or greenish discoloration of the fluid.

Blood is often recognized by simple inspection, but more frequently requires a chemic test. It is bright red when very fresh, and dark, resembling coffee-grounds, when older. Vomiting of blood, or *hematemesis*, may be mistaken for pulmonary hemorrhage, or *hemoptysis*. In the former the fluid is acid in reaction and usually dark red or brown in color and clotted, while in hemoptysis it is brighter red, frothy, alkaline, and usually mixed with a variable amount of mucus.

Particles of food eaten hours or even days previously may be found, and indicate deficient motor power.

Search should always be made for *bits of tissue* from the gastric mucous membrane or new growths. These, when examined by a pathologist, will sometimes render the diagnosis clear.

C. CHEMIC EXAMINATION

A routine chemic examination of the gastric contents involves qualitative tests for free acids, free hydrochloric acid, and organic acids, and quantitative estimations of total acidity, free hydrochloric acid, and sometimes combined hydrochloric acid. Other tests are applied when indicated.

1. Qualitative Tests.—(1) **Free Acids.**—The presence or absence of free acids, without reference to the kind, is easily determined .by means of Congo-red, although the test is not much used in practice.

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Congo-red Test.—Take a few drops of a strong alcoholic solution of Congo-red in a test-tube, dilute with water to a strong red color, and add a few cubic centimeters of filtered gastric juice. The appearance of a *blue color* shows the presence of some free acid (Plate XI, B, B'). Since the test is more sensitive to mineral than to organic acids, a marked reaction points to the presence of free hydrochloric acid.

Thick filter-paper soaked in Congo-red solution, dried, and cut into strips may be used, but the test is much less delicate when thus applied.

(2) **Free Hydrochloric Acid.**—In addition to its digestive function, free hydrochloric acid is an efficient antiseptic. It prevents or retards fermentation and lactic-acid formation, and is an important means of protection against the entrance of pathogenic organisms into the body. It is never absent in health.

Amidobenzol Test.—To a little of the filtered gastric juice in a test-tube, or to several drops in a porcelain dish, add a drop of 0.5 per cent. alcoholic solution of dimethylamidoazobenzol. In the presence of free hydrochloric acid there will at once appear a *cherry-red color*, varying in intensity with the amount of acid (Plate XII, C). This test is very delicate; but, unfortunately, organic acids, when present in large amounts (above 0.5 per cent.), give a similar reaction.

Boas' Test.—This test is less delicate than the preceding, but is more reliable, since it reacts only to free hydrochloric acid.

In a porcelain dish mix a few drops of the gastric juice and the reagent, and slowly evaporate to dryness over a flame, *taking care not to scorch*. The appearance of a *rose-red color*, which fades upon cooling, shows the presence of free hydrochloric acid (Plate XI, 1).

PLATE XI
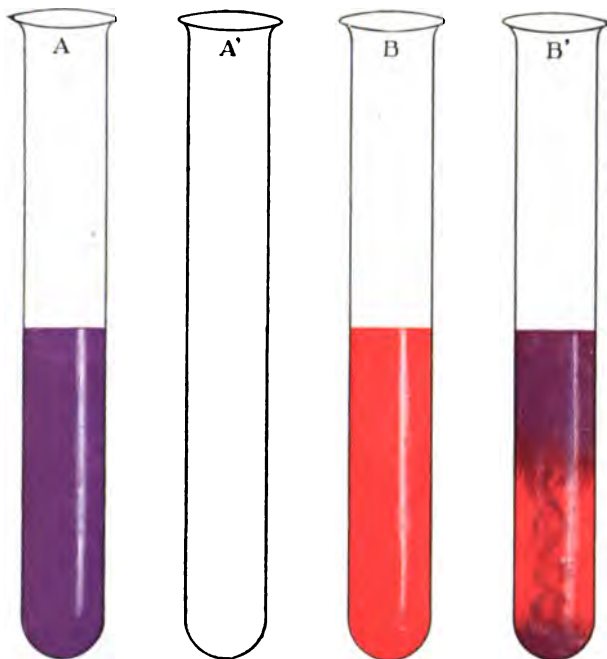
A, Uffelmann's reagent; A', A after the addition of gastric fluid containing lactic acid; B, water to which three drops of Congo-red solution have been added; B', change induced in B when gastric fluid containing free hydrochloric acid is added (Boston).
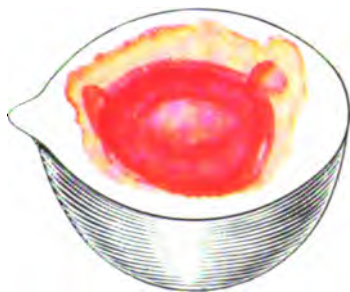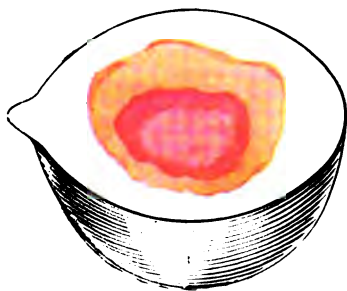
1, Resorcin-test for free hydrochloric acid; 2, Günzburg's test for hydrochloric acid (Boston).

Boas' reagent consists of 5 gm. resublimed resorcinol, and 3 gm. cane-sugar, in 100 c.c. alcohol. The solution keeps well, which, from the practitioner's view-point, makes it preferable to Günzburg's phloroglucin-vanillin reagent (phloroglucin, 2 gm.; vanillin, 1 gm.; absolute alcohol, 30 c.c.). The latter is just as delicate, is applied in the same way, and gives a sharper reaction (Plate XI, 2), but is unstable.

(3) **Organic Acids.**—Lactic acid is the most common, and is taken as the type of the organic acids which appear in the stomach-contents. It is a product of bacterial activity. Acetic and butyric acids are sometimes present. Their formation is closely connected with that of lactic acid, and they are rarely tested for. When abundant, they may be recognized by their odor upon heating.

Lactic acid is never present at the height of digestion in health. Although usually present early in digestion, it disappears when free hydrochloric acid begins to appear. Small amounts may be introduced with the food. Pathologically, small amounts may be present whenever there is stagnation of the gastric contents with deficient hydrochloric acid, as in many cases of dilatation of the stomach and chronic gastritis. The presence of notable amounts of lactic acid (more than 0.1 per cent. by Strauss' test) is strongly suggestive of gastric cancer, and is probably the most valuable single symptom of the disease.

As already stated, the Ewald test-breakfast introduces a small amount of lactic acid, but rarely enough to respond to the tests given here. In every case, however, in which its detection is important, the shredded-wheat biscuit or Boas' test-breakfast should be given, the

stomach having been thoroughly washed the evening before.

Uffelmann's Test for Lactic Acid.—Thoroughly shake up 5 c.c. of filtered stomach fluid with 50 c.c. of ether for at least ten minutes. Collect the ether and evaporate over a water-bath. Dissolve the residue in 5 c.c. water and test with Uffelmann's reagent as follows:

In a test-tube mix 3 drops concentrated solution of phenol and 3 drops saturated aqueous solution of ferric chlorid. Add water until the mixture assumes an amethyst-blue color. To this add the solution to be tested. The appearance of a *canary-yellow color* indicates the presence of lactic acid (Plate XI, A, A').

Uffelmann's test may be applied directly to the stomach-contents without extracting with ether, but is then neither sensitive nor reliable, because of the phosphates, sugars, and other interfering substances which may be present.

Kelling's Test (*Simon's Modification*).—This is much more satisfactory than Uffelmann's. To a test-tube of distilled water add sufficient ferric chlorid solution to give a faint yellowish tinge. Pour half of this into a second test-tube to serve as a control. To the other add a small amount of the gastric juice. Lactic acid gives a distinct yellow color which is readily recognized by comparison with the control.

Strauss' Test for Lactic Acid.—This is a good test for clinical work, since it gives a rough idea of the quantity present and is not sufficiently sensitive to respond to the traces of lactic acid which some test-meals introduce. Strauss' instrument (Fig. 105) is essentially a separating funnel with a mark at 5 c.c. and one at 25 c.c. Fill to the 5 c.c. mark with filtered stomach fluid, and to the 25 c.c. mark with ether. Shake thoroughly for ten or fifteen minutes, let stand until the ether separates, and then, by opening the stop-cock, allow the liquid to run out to the 5 c.c. mark. Fill to the 25 c.c. mark

with water, and add two drops of tincture of ferric chlorid diluted 1 : 10. Shake gently. If 0.1 per cent. or more lactic acid be present, the water will assume a strong greenish-yellow color. A slight tinge will appear with 0.05 per cent.

(4) **Pepsin and Pepsinogen.**—Pepsinogen itself has no digestive power. It is secreted by the gastric glands, and is transformed into pepsin by the action of a free acid. Although pepsin digests proteins best in the presence of free hydrochloric acid, it has a slight digestive activity in the presence of organic or combined hydrochloric acids.

The amount is not influenced by neuroses or circulatory disturbances. Absence or marked diminution, therefore, indicates organic disease of the stomach. It is an important point in diagnosis between functional and organic conditions. Pepsin is rarely or never absent in the presence of free hydrochloric acid.

Fig. 105.—Separatory funnel for Strauss' lactic acid test (Sahli).

Test for Pepsin and Pepsinogen.—With a cork-borer cut small cylinders from the coagulated white of an egg, and cut these into discs of uniform size. The egg should be cooked very slowly, preferably over a water-bath, so that the white may be readily digestible. The discs may be preserved in glycerin, but must be washed in water before using.

Place a disc in each of three test-tubes.

Into tube No. 1 put 10 c.c. distilled water, 5 grains pepsin, U. S. P., and 3 drops of the official dilute hydrochloric acid.

Into tube No. 2 put 10 c.c. filtered gastric juice.

Into tube No. 3 put 10 c.c. filtered gastric juice and 3 drops dilute hydrochloric acid.

Place the tubes in an incubator or warm water for three hours or longer. At intervals, observe the extent to which the egg-albumen has been digested. This is recognized by the depth to which the disc has become translucent.

Tube No. 1 is used for comparison, and should show the effect of normal gastric juice.

Digestion of the egg in tube No. 2 indicates the presence of both pepsin and free hydrochloric acid.

When digestion fails in tube No. 2 and occurs in No. 3, pepsinogen is present, having been transformed into pepsin by the hydrochloric acid added. Should digestion fail in this tube, both pepsin and pepsinogen are absent.

(5) **Rennin and Renninogen.**—Rennin is the milk-curdling ferment of the gastric juice. It is derived from renninogen through the action of hydrochloric acid. Lime salts also possess the power of transforming renninogen into the active ferment.

Deficiency of rennin has the same significance as deficiency of pepsin, and is more easily recognized. Since the two enzymes are almost invariably present or absent together, the test for rennin serves also as a test for pepsin.

Test for Rennin.—Neutralize 5 c.c. filtered gastric juice with very dilute sodium hydroxid solution; add 5 c.c. fresh milk, and place in an incubator or in a vessel of water at about 104° F. Coagulation of the milk in ten to fifteen minutes shows a normal amount of rennin. Delayed coagulation denotes a less amount.

Test for Renninogen.—To 5 c.c. neutralized gastric juice add 2 c.c. of 1 per cent. calcium chlorid solution and 5 c.c. fresh milk, and place in an incubator.   If coagulation occurs, renninogen is present.

(6) **Blood.**—Blood is present in the vomitus in a great variety of conditions.   When found in the fluid removed after a test-meal, it commonly points toward ulcer or carcinoma.   Blood can be detected in nearly one-half of the cases of gastric cancer.   The presence of swallowed blood must be excluded.

Test for Blood in Stomach-contents.—To 10 c.c. of the fluid add a few cubic centimeters of glacial acetic acid and shake the mixture thoroughly with an equal volume of ether. Separate the ether and apply to it the guaiac test (p. 125); or evaporate and apply the hemin test (p. 274) to the residue. When brown particles are present in the fluid, the hemin test should be applied directly to them.

2. Quantitative Tests.—(1) **Total Acidity.**—The acid-reacting substances which contribute to the total acidity are free hydrochloric acid, combined hydrochloric acid, acid salts, mostly phosphates, and, in some pathologic conditions, the organic acids.   The total acidity is normally about 50 to 75 *degrees* (see method below) or, when estimated as hydrochloric acid, about 0.2 to 0.3 *per cent.*

Töpfer's Method for Total Acidity.—In an evaporating dish or small beaker (an " after-dinner " coffee-cup is a very convenient substitute) take 10 c.c. filtered stomach-contents and add three or four drops of the indicator, a 1 per cent. alcoholic solution of phenolphthalein.   When the quantity of

stomach fluid is small, 5 c.c. may be used, but results are less accurate than with a larger amount. Add decinormal solution of sodium hydroxid drop by drop from a buret, until the fluid assumes a rose-red color which does not become deeper upon addition of another drop (Plate XII, A, A'). When this point is reached, all the acid has been neutralized. The end reaction will be sharper if the fluid be saturated with sodium chlorid. A sheet of white paper beneath the beaker facilitates recognition of the color change.

In clinical work the amount of acidity is expressed by the number of cubic centimeters of the decinormal sodium hydroxid solution which would be required to neutralize 100 c.c. of the gastric juice, each cubic centimeter representing one *degree* of acidity. Hence multiply the number of cubic centimeters of decinormal solution required to neutralize the 10 c.c. of stomach fluid by ten. This gives the number of degrees of acidity. The amount may be expressed in terms of hydrochloric acid, if one remember that each degree is equivalent to 0.00365 per cent. hydrochloric acid. Some one suggests that this is the number of days in the year, the last figure, 5, indicating the number of decimal places.

Example.—Suppose that 7 c.c. of decinormal solution were required to bring about the end reaction in 10 c.c. gastric juice; then $7 \times 10 = 70$ *degrees* of acidity; and, expressed in terms of hydrochloric acid, $70 \times 0.00365 = 0.255$ *per cent.*

Preparation of decinormal solution is described in textbooks on chemistry. The practitioner will find it best to have them made by a chemist, or to purchase from a chemic supply house. Preparation of an approximately decinormal solution is described on page 436.

(2) **Hydrochloric Acid.**—After the Ewald and Boas test-breakfasts the amount of free hydrochloric acid varies normally between 25 and 50 degrees, or about 0.1

to 0.2 per cent.   In disease it may go considerably higher or may be absent altogether.

When the amount of free hydrochloric acid is normal, organic disease of the stomach probably does not exist.

Increase of free hydrochloric acid above 50 degrees (*hyperchlorhydria*) generally indicates a neurosis, but also occurs in most cases of gastric ulcer and beginning chronic gastritis.

Decrease of free hydrochloric acid below 25 degrees (*hypochlorhydria*) occurs in some neuroses, chronic gastritis, early carcinoma, and most conditions associated with general systemic depression.   Marked variation in the amount at successive examinations strongly suggests a neurosis.   Too low values are often obtained at the first examination, the patient's dread of the introduction of the tube probably inhibiting secretion.

Absence of free hydrochloric acid (*achlorhydria*) occurs in most cases of gastric cancer and far-advanced chronic gastritis, in many cases of pernicious anemia, and sometimes in hysteria and pulmonary tuberculosis.

The presence of free hydrochloric acid presupposes a normal amount of combined hydrochloric acid, hence the combined need not be estimated when the free acid has been found.   When, however, free hydrochloric acid is absent, it is important to know whether any acid is secreted, and an estimation of the combined acid then becomes of great value.   The normal average after an Ewald breakfast is about 10 to 15 degrees, the quantity depending upon the amount of protein in the test-meal.

Töpfer's Method for Free Hydrochloric Acid.—In a beaker take 10 c.c. filtered stomach fluid and add 4 drops

of the indicator, a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol. A red color instantly appears if free hydro-chloric acid be present. Add decinormal sodium hydroxid solution, drop by drop from a buret, until the last trace of red just disappears, and a canary-yellow color takes its place (Plate XII, C, C'). Read off the number of cubic centimeters of decinormal solution added, and calculate the degrees, or percentage of free hydrochloric acid, as in Töpfer's method for total acidity.

When it is impossible to obtain sufficient fluid for all the tests, it will be found convenient to estimate the free hydro-chloric acid and total acidity in the same portion. After finding the free hydrochloric acid as just described, add 4 drops phenolphthalein solution, and continue the titration. The amount of decinormal solution used in both titrations indicates the total acidity.

Töpfer's Method for Combined Hydrochloric Acid.—In a beaker take 10 c.c. filtered gastric juice and add 4 drops of the indicator, a 1 per cent. aqueous solution of sodium alizarin sulphonate. Titrate with decinormal sodium hy-droxid until the appearance of a bluish-violet color which does not become deeper upon addition of another drop (Plate XII, B, B'). It is difficult, without practice, to determine when the right color has been reached. A reddish-violet appears first. The shade which denotes the end reaction can be produced by adding 2 or 3 drops of the indicator to 5 c.c. of 1 per cent. sodium carbonate solution.

Calculate the number of cubic centimeters of decinormal solution which would be required for 100 c.c. of stomach fluid. This gives, in degrees, *all the acidity except the combined hydro-chloric acid*. The combined hydrochloric acid is then found by deducting this amount from the total acidity, which has been previously determined.

Example.—Suppose that 5 c.c. of decinormal solution were required to produce the purple color in 10 c.c. gastric juice;

PLATE XII
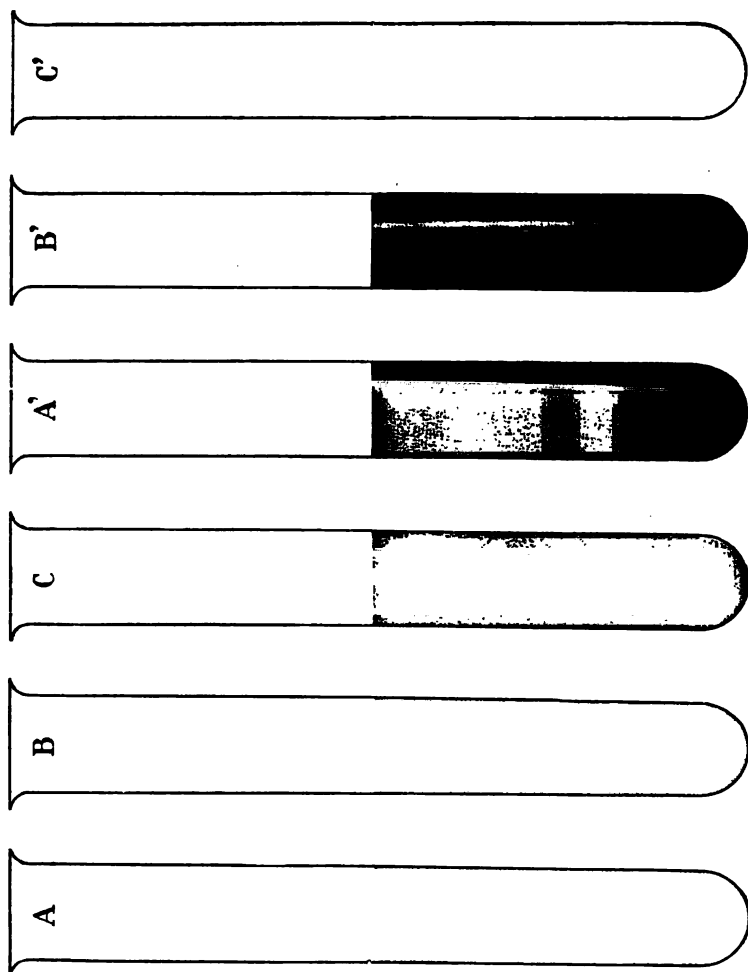
A, Gastric fluid to which a 1 per cent. solution of phenolphthalein has been added; B, gastric fluid to which a 1 per cent. solution of alizarin has been added; C, gastric fluid to which a 0.5 per cent. solution of dimethylamido-azobenzol has been added; A', A after titration with a decinormal solution of sodium hydroxid; B', B after titration with a decinormal solution of sodium hydroxid; C', C after titration with a decinormal solution of sodium hydroxid (Boston).

then $5 \times 10 = 50 = $ *all the acidity except combined hydrochloric acid*. Suppose, now, that the total acidity has already been found to be 70 degrees; then $70 - 50 = 20$ *degrees* of combined hydrochloric acid; and $20 \times 0.00365 = 0.073$ *per cent.*

When free hydrochloric acid is absent, it is probably more helpful to estimate the **acid deficit** than the combined hydrochloric acid. The acid deficit shows how far the acid secreted by the stomach falls short of saturating the protein (and bases) of the meal. It represents the amount of hydrochloric acid which must be added to the fluid before a test for free hydrochloric acid can be obtained. It is determined by titrating with $\frac{n}{10}$ hydrochloric acid, using dimethyl-amido-azo-benzol as indicator, until the fluid assumes a red color. The amount of deficit is expressed by the number of cubic centimeters of the decinormal solution required for 100 c.c. of the stomach fluid.

(3) **Organic Acids.**—There is no simple direct quantitative method. After the total acidity has been determined, organic acids may be removed from another portion of the gastric filtrate by shaking thoroughly with an equal volume of neutral ether, allowing the fluids to separate, and repeating this process until the gastric fluid has been extracted with eight or ten times its volume of ether. The total acidity is then determined, and the difference between the two determinations indicates the amount of organic acids.

(4) **Pepsin.**—No direct method is avaliable. The following is sufficient for clinical purposes:

Hammerschlag's Method.—To the white of an egg add twelve times its volume of 0.4 per cent. hydrochloric acid

(dilute hydrochloric acid, U. S. P., 4 c.c.; water, 96 c.c.), mix well, and filter. This gives a 1 per cent. egg-albumen solution. Take 10 c.c. of this solution in each of three tubes or beakers. To *A* add 5 c.c. gastric juice; to *B*, 5 c.c. water with 0.5 gm. pepsin; to *C*, 5 c.c. water only. Place in an incubator for an hour and then determine the amount of albumin in each mixture by Esbach's method. Tube *C* shows the amount of albumin in the test-solution. The difference between *C* and *B* indicates the amount of albumin which would be digested by normal gastric juice. The difference between *C* and *A* gives the albumin which is digested by the fluid under examination. Schütz has shown that the amounts of pepsin in two fluids are proportionate to the squares of the products of digestion. Thus, if the amounts of albumin digested in tubes *A* and *B* are to each other as 2 is to 4, the amounts of pepsin are to each other as 4 is to 16.

Certain sources of error can be eliminated by diluting the gastric juice several times before testing. The most important of these are that the law of Schütz holds good only for comparatively dilute solutions, and that the products of peptic activity inhibit digestion.

Mett's method is generally preferred to the preceding. Put three or four Mett's tubes about 2 cm. long into a small beaker with diluted gastric juice (1 c.c. of the filtrate plus 15 c.c. twentieth-normal hydrochloric acid). Place in an incubator for twenty-four hours, and then measure as accurately as possible the column which has been digested, using a millimeter scale and a hand lens or, better, a low power of the microscope and an eye-piece micrometer. Square the average length of this column (law of Schütz) and multiply by the degree of dilution, 16. The maximum figure obtained in this way is 256, representing a digested column of 4 mm.

Prepare Mett's tubes as follows:

Beat up slightly the whites of one or two eggs and filter. Pour into a wide test-tube and stand in this a number of

capillary glass tubes, 1 to 2 mm. in diameter. When the tubes are filled, plug their ends with bread crumbs, and coagulate the albumin by heating in water just short of boiling. Dip the ends of the tube in melted paraffin and preserve until needed. Bubbles, if present, will probably disappear in a few days. When wanted for use, cut the tubes into lengths of about 2 cm. Discard any in which the albumin has separated from the wall.

D. Microscopic Examination

A drop of unfiltered stomach-contents is placed upon a slide, covered with a cover-glass, and examined with the 16 mm. and 4 mm. objectives. A drop of Lugol's
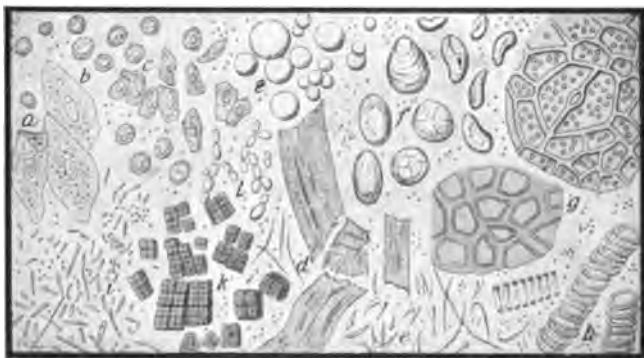
Fig. 106.—General view of the gastric contents: *a*, Squamous epithelial cells from esophagus and mouth; *b*, leukocytes; *c*, cylindric epithelial cells; *d*, muscle-fibers; *e*, fat-droplets and fat-crystals; *f*, starch-granules; *g*, chlorophyl-containing vegetable matters; *h*, vegetable spirals; *i*, bacteria; *k*, sarcinæ; *l*, budding (yeast) fungi (Jakob).

solution allowed to run under the cover will aid in distinguishing the various structures.

Under normal conditions little is to be seen except great numbers of starch-granules, with an occasional

epithelial cell, yeast-cell, or bacterium. Starch-granules are recognized by their concentric striations and the fact that they stain blue with iodin solutions when undigested, and reddish, due to erythrodextrin, when partially digested.

Pathologically, remnants of food from previous meals, red blood-corpuscles, pus-cells, sarcinæ, and excessive numbers of yeast-cells and bacteria may be encountered (Fig. 106).

Remnants of food from previous meals indicate deficient gastric motility.

Red Blood-corpuscles.—Blood is best recognized by the chemic tests already given. The corpuscles sometimes retain a fairly normal appearance, but are generally so degenerated that only granular pigment is left. When only a few fresh looking corpuscles are present, they usually come from irritation of the mucous membrane by the tube.

Pus-cells.—Pus is rarely encountered in the fluid removed after a test-meal. Considerable numbers of pus-corpuscles have been found in some cases of gastric cancer. Swallowed sputum must always be considered.

Sarcinæ.—These are small spheres arranged in cuboid groups, often compared to bales of cotton. They frequently form large clumps and are easily recognized. They stain brown with iodin solution. They signify fermentation. Their presence is strong evidence against the existence of gastric cancer, in which disease they rarely occur.

Yeast-cells.—As already stated, a few yeast-cells may be found under normal conditions. The presence of considerable numbers is evidence of fermentation.

Their appearance has been described (p. 171). They stain brown with iodin solution.

Bacteria.—Numerous bacteria may be encountered, especially in the absence of free hydrochloric acid. The *Boas-Oppler bacillus* is the only one of special significance. It occurs in the majority of cases of cancer, and is rarely found in other conditions. Carcinoma probably furnishes a favorable medium for its growth.

Fig. 107.—Boas-Oppler bacillus from case of gastric cancer (Boston).

These bacilli (Fig. 107) are large (5 to 10 μ long), non-motile, and usually arranged in clumps or end to end in zig-zag chains. They stain brown with iodin solution, which distinguishes them from *Leptothrix buccalis* (p. 377), which is not infrequently found in stomach fluid. They also stain by Gram's method. They are easily seen with the 4 mm. objective in unstained preparations, but are best recognized with the oil lens, after drying some of the fluid upon a cover-glass, fixing, and staining with a simple bacterial stain or by Gram's method.

A few large non-motile bacilli are frequently seen; they cannot be called Boas-Oppler bacilli unless they are numerous and show something of the typical arrangement.

E.   THE GASTRIC CONTENTS IN DISEASE

In the diagnosis of stomach disorders the practitioner must be cautioned against relying too much upon examinations of the stomach-contents.   A first examination is especially unreliable.   Even when repeated examinations are made, the laboratory findings must never be considered apart from the clinical signs.

The more characteristic findings in certain disorders are suggested here.

1. Dilatation of the Stomach.—Evidences of retention and fermentation are the chief characteristics of this condition.   Hydrochloric acid is commonly diminished. Pepsin may be normal or slightly diminished.   Lactic acid may be detected in small amounts, but is usually absent when the stomach has been washed before giving the test-meal.   Both motility and absorptive power are deficient.   The microscope commonly shows sarcinæ, bacteria, and great numbers of yeast-cells.   Remnants of food from previous meals can be detected with the naked eye or microscopically.

2. Gastric Neuroses.—The findings are variable. Successive examinations may show normal, increased, or diminished hydrochloric acid, or even entire absence of the free acid.   Pepsin is usually normal.

In the neurosis characterized by continuous hypersecretion (gastrosuccorrhea), 40 c.c. or more of gastric juice can be obtained from the fasting stomach.   Should

the fluid contain food-particles, it is probably the result of retention, not hypersecretion.

3. Chronic Gastritis.—Free hydrochloric acid may be increased in early cases. It is generally diminished in well-marked cases, and is often absent in advanced cases. Lactic acid is often present in traces, rarely in notable amount. Secretion of pepsin and rennin is always diminished in marked cases. Mucus is frequently present, and is very significant of the disease. Motility and absorption are generally deficient. Small fragments of mucous membrane may be found, and when examined by a pathologist, may occasionally establish the diagnosis.

4. Achylia Gastrica (Atrophic Gastritis).—This condition may be a terminal stage of chronic gastritis. It is sometimes associated with the blood-picture of pernicious anemia. It gives a great decrease, and sometimes entire absence of hydrochloric acid and ferments. The total acidity may be as low as 1 or 2 degrees. Small amounts of lactic acid may be present. Absorption and motility are usually not affected greatly.

5. Gastric Carcinoma.—As far as the laboratory examination goes, the cardinal signs of this disease are absence of free hydrochloric acid and presence of lactic acid and of the Boas-Oppler bacillus. These findings are, however, by no means constant.

It is probable that some substance is produced by the cancer which neutralizes the free hydrochloric acid, and thus causes it to disappear earlier than in other organic diseases of the stomach.

The presence of lactic acid is the most suggestive single symptom of gastric cancer. In the great majority of cases its presence in notable amount (0.1 per cent. by

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Strauss' method) after Boas' breakfast, the stomach having been washed the evening before, warrants a tentative diagnosis of malignancy.

Carcinoma seems to furnish an especially favorable medium for the growth of the Boas-Oppler bacillus, hence this micro-organism is frequently present.

Blood can be detected in the stomach fluid by the chemic tests in nearly one-half of the cases, and is more common when the new growth is situated at the pylorus. Blood is present in the stool in nearly every case.

Evidences of retention and fermentation are the rule in pyloric cancer. Tumor particles are sometimes found late in the disease.

6. Gastric Ulcer.—There is excess of free hydrochloric acid in about one-half of the cases. In other cases the acid is normal or diminished. Blood is often present. The diagnosis must be based largely upon the clinical symptoms, and where ulcer is strongly suspected, it is probably unwise to use the stomach-tube.

II. ADDITIONAL EXAMINATIONS WHICH GIVE INFORMATION AS TO THE CONDITION OF THE STOMACH

1. Absorptive Power of the Stomach.—This is a very unimportant function, only a few substances being absorbed in the stomach. It is delayed in most organic diseases of the stomach, especially in dilatation and carcinoma, but not in neuroses. The test has little practical value.

Give the patient, upon an empty stomach, a 3-grain capsule of potassium iodid with a glass of water, taking care that none of the drug adheres to the outside of the capsule.

At intervals test the saliva for iodids by moistening starch-paper with it and touching with yellow nitric acid. A blue color shows the presence of an iodid, and appears normally in ten to fifteen minutes after ingestion of the capsule. A longer time denotes delayed absorption.

Starch paper is prepared by soaking filter-paper in boiled starch and drying.

2. Motor Power of the Stomach.—This refers to the rapidity with which the stomach passes its contents on into the intestines. It is very important: intestinal diges-tion can compensate for insufficient or absent stomach digestion only so long as the motor power is good.

Motility is impaired to some extent in chronic gastritis. It is especially deficient in dilatation of the stomach due to atony of the gastric wall or to pyloric obstruction, either benign or malignant. It is increased in most con-ditions with hyperchlorhydria.

The best evidence of deficient motor power is the detection of food in the stomach at a time when it should be empty, *e. g.*, before breakfast in the morning. When more than 60 c.c. of fluid are obtained with the tube one hour after a Ewald breakfast, deficient motility may be inferred.

Ewald's salol test is scarcely so reliable as the above. It depends upon the fact that salol is not absorbed until it reaches the intestines and is decomposed by the alkaline intestinal juices.

The patient is given 15 grains of salol with a test-breakfast, and the urine, passed at intervals thereafter, is tested for salicyluric acid. A few drops of 10 per cent. ferric chlorid solution are added to a small quantity of the urine. A violet

color denotes the presence of salicyluric acid. It appears normally in sixty to seventy-five minutes after ingestion of the salol. A longer time indicates impaired motor power.

3. To Determine Size and Position of Stomach.— After removing the test-meal, while the tube is still in place, force quick puffs of air into the stomach by compression of the bulb. The puffs can be clearly heard with a stethoscope over the region of the stomach, and nowhere else.

If desired, the patient may be given a dram of sodium bicarbonate in solution, followed immediately by the same amount of tartaric acid, also in solution; or he may take the two parts of a seidlitz powder separately. The carbon dioxid evolved distends the stomach, and its outline can easily be determined by percussion.

4. Sahli's Desmoid Test of Gastric Digestion.—Two pills, one containing 0.1 gram iodoform, the other 0.05 gram methylene-blue, are wrapped in little bags made of thin sheets of rubber and tied with a string of catgut. The bags must be carefully folded and tied. For detailed directions the reader is referred to Sahli's book, Diagnostic Methods.

The patient swallows the two bags with the aid of a little water during the noon meal, and the urine is tested at intervals thereafter. According to Sahli, the catgut is digested by gastric juice and not by pancreatic or intestinal juices. If gastric digestion is normal, iodin and methylene-blue can be detected in the urine in the afternoon or evening of the same day. The reaction may occur when digestion is very poor, provided gastric motility is diminished, but it is then delayed. If the

reaction does not appear, gastric digestion has not occurred.

Methylene-blue is recognized in the urine by the green or blue color which it imparts. It is sometimes eliminated as a chromogen; and a little of the urine must be acidified with acetic acid and boiled to bring out the color.

To detect the iodin, some of the urine is decolorized by gently heating and filtering through animal charcoal. To 10 c.c. are then added 1 c.c. dilute sulphuric acid, and 0.5 c.c. of a 1 per cent. solution of sodium nitrite and 2 c.c. of chloroform. Upon shaking, a rose color will be imparted to the chloroform if iodin be present.

CHAPTER V

THE FECES

As commonly practised, an examination of the feces is limited to a search for intestinal parasites or their ova. Much of value can, however, be learned from other simple examinations, particularly a careful inspection. Anything approaching a complete analysis is, on the other hand, a waste of time for the clinician.

The normal stool is a mixture of—(a) water; (b) undigested and indigestible remnants of food, as starch-granules, particles of meat, plant-cells and fibers, etc.; (c) digested foods, carried out before absorption could take place; (d) products of the digestive tract, as altered bile-pigments, mucus, etc.; (e) products of decomposition, as indol, skatol, fatty acids, and various gases; (f) epithelial cells shed from the wall of the intestinal canal; (g) harmless bacteria, which are always present in enormous numbers.

Pathologically, we may find abnormal amounts of normal constituents, blood, pathogenic bacteria, animal parasites and their ova, and biliary and intestinal concretions.

The stool to be examined should be passed into a clean vessel, without admixture of urine. The offensive odor can be partially overcome with turpentine or 5 per cent. phenol. When search for amebae is to be made, the vessel must be warm, and the stool kept warm until examined; naturally, no disinfectant can be used.

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I. MACROSCOPIC EXAMINATION

1. Quantity.—The amount varies greatly with diet and other factors.   The average is about 100 to 150 gm. in twenty-four hours.

2. Frequency.—One or two stools in twenty-four hours may be considered normal, yet one in three or four days is not uncommon with healthy persons.   The individual habit should be considered in every case.

3. Form and Consistence.—Soft, mushy, or liquid stools follow cathartics and accompany diarrhea.   Copious, purely serous discharges without fecal matter are significant of Asiatic cholera, although sometimes observed in other conditions.   Hard stools accompany constipation.   Rounded scybalous masses are common in habitual constipation, and indicate atony of the muscular coat of the intestines.   Flattened, ribbon-like stools result from some obstruction in the rectum, generally a tumor or stricture from a healed ulcer, most commonly syphilitic.   When bleeding piles are absent, blood-streaks upon such a stool point to carcinoma.

4. Color.—The normal light or dark-brown color is due chiefly to hydrobilirubin, which is formed from bilirubin by reducing processes in the intestines, largely the result of bacterial activity.   The stools of infants are yellow, owing partly to their milk diet and partly to the presence of unchanged bilirubin.

Diet and drugs cause marked changes: milk, a light yellow color; cocoa and chocolate, dark gray; various fruits, reddish or black; iron and bismuth, dark brown or black; hematoxylin, red, etc.

Pathologically, the color is important.   A golden yellow

is generally due to unchanged bilirubin. Green stools
are not uncommon, especially in diarrheas of childhood.
The color is due to biliverdin or, sometimes, to chromo-
genic bacteria. Putty-colored or " acholic " stools occur
when bile is deficient, either from obstruction to outflow
or from deficient secretion. The color is due less to
absence of bile-pigments than to presence of fat. Similar
stools are common in conditions like tuberculous peri-
tonitis, which interfere with absorption of fats, and in
pancreatic disease.

Notable amounts of blood produce tarry black stools
when the source of the hemorrhage is the stomach or
upper intestine, and a dark brown or bright red as the
source is nearer the rectum. When diarrhea exists the
color may be red, even if the source of the blood is high
up. Red streaks of blood upon the outside of the stool
are due to lesions of rectum or anus.

5. Odor.—Products of decomposition, chiefly indol
and skatol, are responsible for the normal offensive odor.
A sour odor is normal for nursing infants, and is noted in
mild diarrheas of older children. In the severe diarrheas
of childhood a putrid odor is common. In adults, stools
emitting a very foul stench are suggestive of malignant
or syphilitic ulceration of the rectum or gangrenous
dysentery.

6. Mucus.—Excessive quantities of mucus are easily
detected with the naked eye, and signify irritation or
inflammation. When the mucus is small in amount and
intimately mixed with the stool, the trouble is probably
in the small intestine. Larger amounts, not well mixed
with fecal matter, indicate inflammation of the large
intestine. Stools composed almost wholly of mucus and

streaked with blood are the rule in dysentery, ileocolitis, and intussusception.

In the so-called mucous colic or membranous enteritis shreds and ribbons of altered mucus, sometimes representing complete casts of portions of the bowel, are passed. The mucus sometimes takes the form of frog-spawn-like masses. In some cases it is passed at variable intervals, with colic; in others, with every stool, with only vague pains and discomfort. It is distinguished from inflammatory mucus by absence of pus-corpuscles. The condition is not uncommon and should be more frequently recognized. It is probably a secretory neurosis, hence the name " membranous enteritis " is inappropriate.

7. Concretions.—Gall-stones are probably more common than is generally supposed, and should be searched for in every case of obscure colicky abdominal pain. Intestinal concretions (enteroliths) are rare. Intestinal sand, consisting of sand-like grains, is especially common in neurotic conditions, such as mucous colitis.

Concretions can be found by breaking up the fecal matter in a sieve (which may be improvised from gauze) while pouring water over it. It must be remembered that gall-stones, if soft, may go to pieces in the bowel.

8. Animal Parasites.—Segments of tapeworms and the adults and larvæ of other parasites are often found in the stool. They are best searched for in the manner described for concretions. The search should be preceded by a vermicide and a brisk purge. Patients frequently mistake vegetable tissue (long fibers from poorly masticated celery or " greens," cells from oranges, etc.) for intestinal parasites, and the writer has known physicians to make similar mistakes. Even slight famil-

iarity with the microscopic structure of vegetable tissue will prevent the chagrin of such errors.

9. Curds.—The stools of nursing infants frequently contain whitish curd-like masses, due either to imperfect digestion of fat or casein or to excess of these in the diet. When composed of fat, the masses are soluble in ether, and give the Sudan III test. If composed of casein, they will become tough and fibrous-like when placed in formalin (10 per cent.) for twenty-four hours.

II. CHEMIC EXAMINATION

Complicated chemic examinations are of little value to the clinician. Certain tests are, however, important.

1. Blood.—When present in large amount blood produces such changes in the appearance of the stool that it is not likely to be overlooked. Traces of blood (occult hemorrhage) can be detected only by special tests. Recognition of occult hemorrhage has its greatest value in diagnosis of gastric cancer and ulcer. It is constantly present in practically every case of gastric cancer, and is always present, although usually intermittently, in ulcer. Traces of blood also accompany malignant disease of the bowel, the presence of certain intestinal parasites, and other conditions.

Detection of Occult Hemorrhage.—Soften a portion of the stool with water, shake with an equal volume of ether to remove fat, and discard the ether. Treat the remaining material with about one-third its volume of glacial acetic acid and extract with ether. Should the ether not separate well, add a little alcohol. Apply the guaiac test to the ether as already described (p. 125).

In every case iron-containing medicines must be stopped, and blood-pigment must be excluded from the food by giving an appropriate diet, *e. g.*, bread, milk, eggs, and fruit. At the beginning of the restricted diet give a dram of powdered charcoal, or 7 grains of carmin, so as to mark the corresponding stool.

2. Bile.—Normally, unaltered bile-pigment is never present in the feces of adults. In catarrhal conditions of the small intestine bilirubin may be carried through unchanged. It may be demonstrated by filtering (after mixing with water if the stool be solid) and testing the filtrate by Gmelin's method, as described under The Urine.

Hydrobilirubin will give a red color if a little of the stool be rubbed up with saturated mercuric chlorid solution and allowed to stand twenty-four hours. The red color is likewise imparted to microscopic structures which are stained with hydrobilirubin. A green color in this test shows the presence of unchanged bilirubin.

III. MICROSCOPIC EXAMINATION

Care must be exercised in selection of portions for examination. A random search will often reveal nothing of interest. A small bit of the stool, or any suspicious-looking particle, is placed upon a slide, softened with water if necessary, and pressed out into a thin layer with a cover-glass. A large slide—about 2 by 3 inches—with a correspondingly large cover will be found convenient. Most of the structures which it is desired to see can be found with a 16 mm. objective. Details of structure must be studied with a higher power.

The bulk of the stool consists of granular débris. Among the recognizable structures met in normal and pathologic conditions are: Remnants of food, epithelial cells, pus-corpuscles, red blood-corpuscles, crystals, bacteria, and ova of animal parasites (Fig. 108).

1. Remnants of Food.—These include a great variety of structures which are very confusing to the student. Considerable study of normal feces is necessary for their recognition.
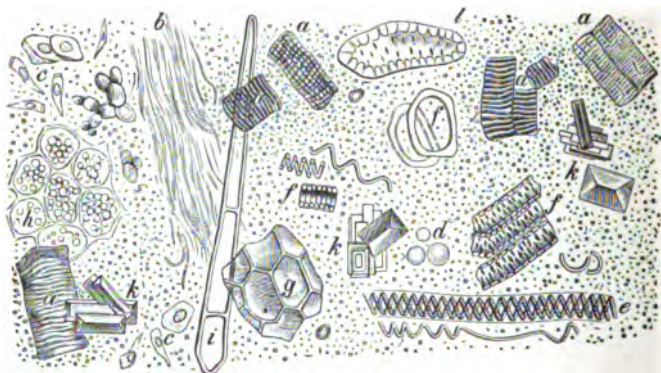
Fig. 108.—Microscopic elements of normal feces: *a*, Muscle-fibers; *b*, connective tissue; *c*, epithelial cells; *d*, white blood-corpuscles; *e*, spiral vessels of plants; *f*–*k*, vegetable cells; *i*, plant hairs; *k*, triple phosphate crystals; *l*, stone cells. Scattered among these elements are micro-organisms and débris (after v. Jaksch).

Vegetable fibers are generally recognized from their spiral structure or their pits, dots, or reticulate markings; *vegetable cells*, from their double contour and the chlorophyl bodies which many of them contain. These cells are apt to be mistaken for the ova of parasites. *Starch-granules* sometimes retain their original form, but are ordinarily not to be recognized except by their staining reaction. They strike a blue color with Lugol's solu-

tion when undigested; a red color, when slightly digested. *Muscle-fibers* are yellow, and when poorly digested appear as short, transversely striated cylinders with rather squarely broken ends (Fig. 109). Generally, the ends are rounded and the striations faint, or only irregularly round or oval yellow masses are found. *Curds of milk* are especially important in the stools of children. They must be distinguished from small masses of *fat* (p. 314).

Fig. 109.—Poorly digested muscle-fiber in feces showing striations (× 200) (photograph by the author).

Excess of any of these structures may result from excessive ingestion or deficient intestinal digestion.

2. Epithelial Cells.—A few cells derived from the wall of the alimentary canal are a constant finding. They show all stages of degeneration, and are often unrecognizable. A marked excess has its origin in a catarrhal condition of some part of the bowel. Squamous cells come from the anal orifice; otherwise the form of the cells gives no clue to the location of the lesion.

3. Pus.—Amounts of pus sufficient to be recognized with the eye alone indicate rupture of an abscess into the bowel. If well mixed with the stool, the source is high up, but in such cases the pus is apt to be more or less completely digested, and hence unrecognizable. Small amounts, detected only by the microscope, are present in catarrhal and ulcerative conditions of the intestine, the number of pus-cells corresponding to the severity and extent of the process.

4. Blood-corpuscles.—Unaltered red corpuscles are rarely found unless their source is near the anus. Ordinarily, only masses of blood-pigment can be seen. Blood is best recognized by the chemic tests (p. 274).

5. Bacteria.—In health, bacteria constitute about one-third of the weight of the dried stool. They are beneficial to the organism, although not actually necessary to its existence. It is both difficult and unprofitable to identify them. The great majority belong to the colon bacillus group, and are negative to Gram's method of staining.

In some pathologic conditions the character of the intestinal flora changes, so that Gram-staining bacteria very greatly predominate. As shown by R. Schmidt, of Neusser's clinic in Vienna, this change is most constant and most striking in cancer of the stomach, owing to large numbers of Boas-Oppler bacilli, and is of considerable value in diagnosis. He believes that a diagnosis of gastric carcinoma should be very unwillingly made with an exclusively "Gram-negative" stool, while a "Gram-positive" stool, due to bacilli (which should also stain brown with Lugol's solution), may be taken as very strong evidence of cancer. A Gram-positive stool due

to cocci is suggestive of intestinal ulceration. The technic is the same as when Gram's method is applied to other material (p. 409), except that the smear is fixed by immersion in methyl-alcohol for five minutes instead of by heat. Fuchsin is the best counterstain. The deep purple Gram-staining bacteria stand out much more prominently than the pale-red Gram-negative organisms, and one may be misled into thinking them more numerous even in cases in which they are much in the minority. The number of Boas-Oppler bacilli can be increased by administering a few ounces of sugar of milk the day before the examination. The bacteria can be obtained comparatively free from food remnants by mixing a little of the feces with water, allowing to settle for a short time, and making smears from the supernatant fluid.

Owing to the difficulty of excluding swallowed sputum, the presence of the tubercle bacillus is less significant in the feces than in other material. It may, however, be taken as evidence of intestinal tuberculosis when clinical signs indicate an intestinal lesion and reasonable care is exercised in regard to the sputum. Success in the search will depend largely upon careful selection of the portion examined. A random search will almost surely fail. Whitish or grayish flakes of mucus or blood-stained or purulent particles should be spread upon slides or covers and stained by the method given upon p. 168. In the case of rectal ulcers, swabs can be made directly from the ulcerated surface.

6. **Crystals.**—Various crystals may be found, but few have any significance. Slender, needle-like crystals of fatty acids and soaps (Fig. 36) and triple phosphate

crystals (Fig. 108) are common. Characteristic octahedral crystals of calcium oxalate (Fig. 51) appear after ingestion of certain vegetables. Charcot-Leyden crystals (Fig. 9) are not infrequently encountered, and strongly suggest the presence of intestinal parasites. Yellowish or brown, needle-like or rhombic crystals of hematoidin (Fig. 36) may be seen after hemorrhage into the bowel.

7. Parasites and Ova.—The stool should be well mixed with water and allowed to settle. The ova will be found in the upper or middle portions of the sediment. The flagellates are best found in the liquid stool after a dose of salts. Descriptions will be found in the following chapter.

IV. FUNCTIONAL TESTS

1. Schmidt's Test Diet.—Much can be learned of the various digestive functions from a microscopic study of the feces, especially when the patient is upon a known diet. For this purpose the standard diet of Schmidt is generally adopted. This consists of:

Morning.......o.5 liter milk and 50 gm. toast.

Forenoon.......o.5 liter porridge, made as follows: 40 gm. oatmeal, 10 gm. butter, 200 c.c. milk, 300 c.c. water, and one egg.

Midday........125 gm. hashed meat, with 20 gm. butter, fried so that the interior is quite rare; 250 gm. potato, made by cooking 190 gm. potato with 100 c.c. milk and 10 gm. butter, the whole boiled down to 250 c.c.

Afternoon......Same as morning.

Evening........Same as forenoon.

At the beginning of the diet, the stool should be marked off with carmin or charcoal. One should famil-

iarize himself with the microscopic appearance of the feces of normal persons upon this diet.

Deficiency of starch digestion is recognized by the number of starch-granules which strike a blue color with iodin. With exception of those inclosed in plant cells none are present normally.

The degree of protein digestion is ascertained by the appearance of the muscle-fibers. Striations are clearly visible only when digestion is imperfect (Fig. 109). According to Schmidt, the presence of nuclei in muscle-fibers denotes complete absence of pancreatic function. The presence of connective-tissue shreds indicates deficient gastric digestion, since raw connective tissue is digested only in the stomach. These shreds can be recognized macroscopically by examining in a thin layer against a black background, and microscopically by their fibrous structure and the fact that they clear up when treated with acetic acid.

Digestion of fats is checked up by the amount of neutral fat.

2. Sahli's Glutoid Test.—The Schmidt test diet involves some inconvenience for the patient, and interpretation of results requires much experience upon the part of the physician. A number of other methods of testing the digestive functions have been proposed. The glutoid test of Sahli is one of the most satisfactory. This is similar to his desmoid test of gastric digestion described on page 308. A glutoid capsule containing 0.15 gram iodoform is taken with an Ewald breakfast. The capsule is not digested by the stomach fluid, but is readily digested by pancreatic juice. Appearance of iodin in the saliva and urine within four to six hours

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indicates normal gastric motility, normal intestinal digestion, and normal absorption. Instead of iodoform, 0.5 gram salol may be used, salicyluric acid appearing in the urine in about the same time. For tests for iodin and salicyluric acid, see pages 307 and 309.

The glutoid capsules are prepared by soaking gelatin capsules in formalin. Sahli states that filled capsules can be purchased of A. G. Haussmann, in St. Gall, Switzerland.

3. Müller's Test for Trypsin.—A calomel purge is given two hours after a meal. Particles of the feces are placed upon solidified blood-serum. This is incubated at a temperature of 55° to 60° C. to prevent action of bacteria. Digestion of the serum—indicated by a translucent, roughened, depressed surface—presumably shows the presence of trypsin, and indicates pancreatic sufficiency. Trypsin can seldom be detected without the preceding purge.

CHAPTER VI

ANIMAL PARASITES

ANIMAL parasites are common in all countries, but are especially abundant in the tropics, where, in some places, almost every native is host for one or more species. Because of our growing intercourse with these regions the subject is assuming increasing importance in this country. Many parasites, hitherto comparatively unknown here, will probably become more common.

Some parasites produce no symptoms, even when present in large numbers. Others cause very serious symptoms. It is, however, impossible to make a sharp distinction between pathogenic and non-pathogenic varieties. Parasites which cause no apparent ill effects in one individual may, under certain conditions, produce marked disturbances in another. The disturbances are so varied, and frequently so indefinite, that diagnosis can rarely be made from the clinical symptoms. It must rest upon detection, by the naked eye or the microscope, of (a) the parasites themselves, (b) their ova or young progeny, or (c) some of their products.

Unlike bacteria, the great majority of animal parasites multiply by means of alternating and differently formed generations, which require widely different conditions for their development. The few exceptions are chiefly among the protozoa. Multiplication of parasites within

the same host is thus prevented. In the case of the hook-worm, for example, there is no increase in the number of worms in the host's intestine, except through re-infection from the outside. The young are carried out of the intestine and must pass a certain period of development in warm, moist earth before they can again enter the human body and grow to maturity. In general, this alternation of periods of development takes place in one of three ways:

(1) The young remain within the original host, but travel to other organs, where they do not reach maturity, but lie quiescent until taken in by a new host. A good example is *Trichinella spiralis*.

(2) The young or the ova which subsequently hatch pass out of the host, and either (*a*) go through a simple process of growth and development before entering another host, as is the case with the hook-worm, or (*b*) pass through one or more free-living generations, the progeny of which infect new hosts, as is the case with *Strongyloides intestinalis*.

(3) The young or ova or certain specialized forms either directly (*e. g.*, malarial parasites) or indirectly (*e. g.*, tapeworms) reach a second host of different species, where a widely different process of development occurs. The host in which the adult or sexual existence is passed is called the *definitive* or final host; that in which the intermediate or larval stage occurs, the *intermediate* host. Man, for example, is the definitive host for *Tænia saginata*, and the intermediate host for the malarial parasites and *Tænia echinococcus*.

A few words concerning the classification and nomenclature of living organisms in general will be helpful

at this place. Individuals which are alike *in all essential respects* are classed together as a *species*. Closely related species are grouped together to form a *genus;* genera which have certain characteristics in common make up a *family;* families are grouped into *orders;* orders into *classes;* and classes, finally, into the *branches* or *phyla*, which make up the *kingdom*. In some cases these groups are subdivided into intermediate groups—subphyla, subfamilies, etc., and occasionally, slight differences warrant subdivision of the species into *varieties*. The animal kingdom comprises nine branches: Protozoa, Porifera, Cœlenterata, Echinodermata, Vermidea, Arthropoda, Mollusca, Prochordata, and Chordata.

The scientific name of an animal or plant consists of two parts, both Latin or Latinized words, and is printed in italics. The first part is the name of the genus and begins with a capital letter; the second is the name of the species and begins with a lower case letter, even when it was originally a proper name. When there are varieties of a species, a third part, the designation of the variety, is appended. The author of the name is sometimes indicated in Roman type immediately after the name of the species. Examples: *Spirochæta vincenti*, often abbreviated to *Sp. vincenti* when the genus name has been used just previously; *Staphylococcus pyogenes albus; Necator americanus*, Stiles.

At the present time there is great confusion in the naming and classification of parasites. Some have been given a very large number of names by different observers, and in many cases different parasites have been described under the same name. The alternation of generations and the marked differences in some cases between male

and female have contributed to the confusion, different forms of the same parasite being described as totally unrelated species.

The number of parasites which have been described as occurring in man and the animals is extremely large. Only those which are of medical interest are mentioned here. They belong to three phyla—Protozoa, Vermidea, and Arthropoda.

PHYLUM PROTOZOA

These are unicellular organisms, the simplest types of animal life. There is very little differentiation of structure. Each contains at least one, and some several nuclei. Some contain contractile vacuoles; some have cilia or flagella as special organs of locomotion. They reproduce by division, by budding, or by sporulation. Sometimes there is an alternation of generations, in one of which sexual processes appear, as is the case with the malarial parasites. The protozoa are very numerous, the subphylum Sarcodina alone including no less than 5000 species. Most of the protozoa are microscopic in size; a few are barely visible to the naked eye. One can gain a general idea of their appearance by examining water (together with a little of the sediment) from the bottom of any pond. Such water usually contains amebæ and a considerable variety of ciliated and flagellated forms.

The following is an outline of those protozoa which are of medical interest, together with the subphyla and classes to which they belong.

PHYLUM PROTOZOA

SUBPHYLUM I. **SARCODINA.**—Locomotion by means of pseudopodia.

CLASS **Rhizopoda.**—Pseudopodia form lobose or reticulose processes.

Genus.	*Species.*
Entamœba.	E. histolytica.
	E. tetragena.
	E. coli.
	E. buccalis.

SUBPHYLUM II. **MASTIGOPHORA (FLAGELLATA).**—Locomotion by means of flagella.

CLASS **Zoömastigophora.**—Forms in which animal characteristics predominate.

Genus.	*Species.*
Spirochæta.	Sp. obermeieri.
	Sp. vincenti.
	Sp. buccalis.
	Sp. dentium.
	Sp. refringens.
Treponema.	T. pallidum.
	T. pertenue.
Trypanosoma.	T. gambiense.
	T. cruzi.
	T. lewisi.
	T. evansi.
	T. brucei.
	T. equiperdum.
Leishmania.	L. donovani.
	L. tropica.
	L. infantum.
Cercomonas.	C. hominis.
Bodo.	B. urinarius.
Trichomonas.	T. vaginalis.
	T. intestinalis.
	T. pulmonalis.
Lamblia.	L. intestinalis.

SUBPHYLUM III.   SPOROZOA.—All members parasitic.   Propagation by means of spores.   No special organs of locomotion.

CLASS Telosporidia.—Sporulation ends the life of the individual.

Genus.	Species.
Coccidium.	C. cuniculi.
Plasmodium.	P. vivax.
	P. malariæ.
	P. falciparum.
Babesia.	B. bigeminum.

SUBPHYLUM IV.   INFUSORIA.—Locomotion by means of cilia.

CLASS Ciliata.—Cilia present throughout life.

Genus.	Species.
Balantidium.	B. coli.

SUBPHYLUM SARCODINA

Class Rhizopoda

These are protozoa the body substance of which forms changeable protoplasmic processes, or pseudopodia, for the taking in of food and for locomotion. They possess one or several nuclei.

1. Genus Entamœba.—(1) Entamœba Histolytica.— This organism is found, often in large numbers, in the stools of tropical dysentery and in the pus and walls of hepatic abscesses associated with dysentery, and is generally regarded as the cause of the disease. It is a colorless, granular cell, 20 to 40 μ in diameter (Fig. 110). It contains one or more distinct vacuoles, a round nucleus, which ordinarily is obscured by the granules, and frequently red blood-corpuscles and bacteria. When at rest its shape is spheric, but upon a warm slide it exhibits the characteristic ameboid motion, constantly changing its shape or moving slowly about. This motion is its most distinctive feature. If neutral red in 0.5 per cent. solution be run under the cover-glass,

it will be taken up by the amebæ and other protozoa and render them conspicuous without killing them ("vital staining").

When the presence of amebæ is suspected, the stool should be passed into a warm vessel and kept warm until and during the examination. A warm stage can be improvised from a plate of copper with a hole cut in the center. This is placed upon the stage of the microscope, and one of the projecting ends is heated with

Fig. 110.—Amœba coli in intestinal mucus, with blood-corpuscles and bacteria (Lösch).

a small flame. Amebæ are most likely to be found in grayish or blood-streaked particles of mucus. Favorable material for examination can be obtained at one's convenience by inserting into the rectum a large catheter with roughly cut lateral openings. A sufficient amount of mucus or fecal matter will usually be brought away by it.

(2) **Other Entamebæ.**—*Entamœba coli*, a similar but somewhat smaller organism (10 to 20 μ), with less dis-

tinct pseudopodia· and more distinct nucleus, has frequently been found in the stools of healthy persons. *E. tetragena* has recently been described. It apparently produces a chronic diarrhea and is not confined to the tropics. Another, *E. buccalis*, has been found in decaying teeth A number of similar organisms have been described as occurring in pus and in ascitic and other body fluids, but it is probable that in many cases, at least, the structures seen were ameboid body cells.

SUBPHYLUM MASTIGOPHORA (FLAGELLATA)
Class Zoömastigophora

The protozoa of this subphylum are provided with one or several whip-like appendages with lashing motion, termed flagella, which serve for locomotion and, in some cases, for feeding. They generally arise from the anterior part of the organism. Some members of the group also possess an undulating membrane—a delicate membranous fold which extends the length of the body, and somewhat suggests a fin. When in active motion this gives the impression of a row of cilia. The flagellata do not exhibit ameboid motion, and, in general, maintain an unchanging oval or spindle shape, and contain a single nucleus. The cytoplasm contains numerous granules and usually several vacuoles, one or more of which may be contractile. Encystment as a means of resisting unfavorable conditions is common.

1. Genus Spirochæta.—The spirochætæ appear to occupy a position midway between the bacteria and protozoa, but are more frequently described with the latter.

(1) **Spirochæta Recurrentis.**—This spirochæte was

described by Obermeier as the cause of relapsing fever. It appears in the circulating blood during the febrile attack, and, unlike the malarial parasite, lives in the plasma without attacking the red corpuscles. The organism is an actively motile spiral, 16 to 40 μ long, with three to twelve wide, fairly regular turns. It can be seen in fresh unstained blood with a high dry lens, being located by the commotion which it creates among

Fig. 111.—Spirochæte of relapsing fever ($\times$ 1000) (Karg and Schmorl).

the red cells. For diagnosis, thin films, stained with Wright's or some similar blood-stain, are used (Fig. 111).

Besides *Spirochæta recurrentis*, a number of distinct strains have been described in connection with different types of relapsing fever: *Sp. novyi* (Plate VII), *Sp. kochi*, *Sp. duttoni*, and *Sp. carteri*.

(2) **Spirochæta Vincenti.**—In stained smears from the ulcers of Vincent's angina (p. 380) are found what appear to be two organisms. One, the " fusiform bacil-

lus," is a slender rod, 6 to 12 μ long, pointed at both ends and sometimes curved. The other is a slender spiral organism, 30 to 40 μ long, with three to eleven comparatively shallow turns (Fig. 153). These were formerly thought to be bacteria, a spirillum and bacillus living in symbiosis. The present tendency is to regard them as stages or forms of the same organism, and to class them among the spirochætæ. The same organisms are quite constantly present in large numbers in ulcerative stomatitis and in noma. They are not infrequently found in small numbers in normal mouths.
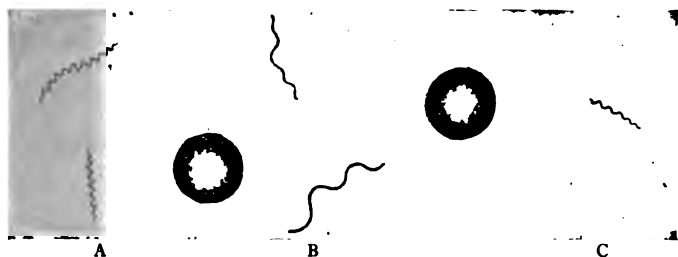
Fig. 112.—Spiral organisms: A, Treponema pallidum; B, Spirochæta refringens; C, Spirochæta dentium. Two red corpuscles are also shown (× 1200).

(3) **Other Spirochætæ.**—A number of harmless forms are of interest because of the possibility of confusing them with the more important pathogenic varieties. Of these, *Sp. buccalis* and *Sp. dentium* are inhabitants of the normal mouth. The former is similar in morphology to *Sp. vincenti*. *Sp. dentium* (Fig. 112) is smaller, more delicate, has deep curves, and may be easily mistaken for *Treponema pallidum*. It, also, stains reddish with Giemsa's stain. In suspected syphilitic sores of the mouth it is, therefore, important to make smears

from the tissue juices rather than from the surface (see p. 389). *Sp. refringens* is frequently present upon the surface of ulcers, especially about the genitals, and has doubtless many times been mistaken for *Treponema pallidum*. It can be avoided by properly securing the material for examination; but its morphology should be sufficient to prevent confusion. It is thicker than the organism of syphilis, stains more deeply, and has fewer and shallower curves (Fig. 112). Giemsa's stain gives it a bluish color.

2. Genus Treponema.—(1) **Treponema Pallidum.**— This is the organism of syphilis. Its description and methods of diagnosis will be found on p. 388.

(2) **Treponema pertenue,** morphologically very similar to *Treponema pallidum*, was found by Castellani in yaws, a skin disease of the tropics.

3. Genus Trypanosoma.—Trypanosomes have been found in the blood-plasma of a great variety of vertebrates. Many of them appear to produce no symptoms, but a few are of great pathologic importance. As seen in the blood, they are elongated, spindle-shaped bodies, the average length of different species varying from 10 to 70 μ. Along one side there runs a delicate undulating membrane, the free edge of which appears to be somewhat longer than the attached edge, thus throwing it into folds. Somewhere in the body, usually near the middle, is a comparatively pale-staining nucleus; and near the posterior end is a smaller, more deeply staining chromatin mass, the micronucleus or blepharoplast. A number of coarse, deeply staining granules, chromatophores, may be scattered through the cytoplasm. A flagellum arises in the blepharoplast, passes along the free edge of the

undulating membrane, and is continued anteriorly as a free flagellum. These details of structure are well shown in Plate VII.

The life history of the trypanosomes is not well known. In most cases there is an alternation of hosts, various insects playing the part of definitive host.

Trypanosomes have been much studied of late, and many species have been described. Of these, only a few
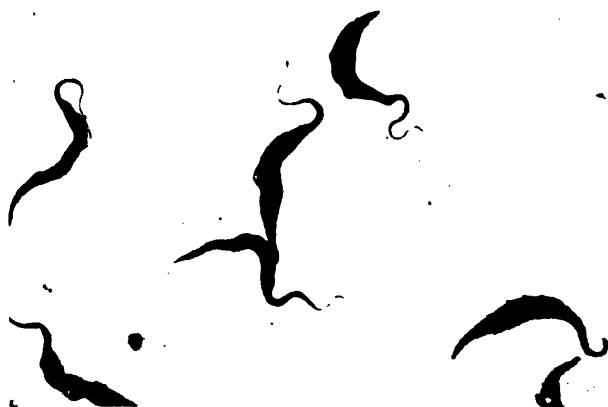
Fig. 113.—Trypanosoma lewisi in blood of rat. The red corpuscles were decolorized with acetic acid (× 1000) (photograph by the author from a slide presented by Prof. Novy).

have medical interest. At least two have been found in man.

Trypanosoma gambiense is the parasite of African "sleeping sickness." Its detection in the blood is described on p. 247.

Trypanosoma cruzi is a small form which has been found in the blood of man in Brazil.

Trypanosoma lewisi, a very common and apparently harmless parasite of gray rats, especially sewer rats, is

interesting because it closely resembles the pathogenic forms, and is easily obtained for study. Its posterior end is more pointed than that of *T. gambiense.*

Trypanosoma evansi, T. brucei, and *T. equiperdum* produce respectively surra, nagana, and dourine, which are common and important diseases of horses, mules, and cattle in the Philippines, East India, and Africa.

4. Genus Leishmania.—The several species which compose this genus are apparently closely related to the trypanosomes, but their exact classification is undetermined. They have been grown outside the body and their transformation into flagellated trypanosome-like structures has been demonstrated. Calkins places them in the genus *Herpetomonas.*

(1) **Leishmania donovani** is the cause of kala-azar, an important and common disease of India. The "Leishman-Donovan bodies" are round or oval structures, 2 to 3 μ in diameter, with two distinct chromatin masses, one large and pale, the other small and deeply staining. The parasites are especially abundant in the spleen, splenic puncture being resorted to for diagnosis. They are readily found in smears stained by any of the Romanowsky methods. They lie chiefly within endothelial cells and leukocytes They are also present within leukocytes in the peripheral blood, but are difficult to find in blood-smears.

(2) **Leishmania tropica** resembles the preceding. It is found, lying intracellularly, in the granulation tissue of Delhi boil or Oriental sore.

(3) **Leishmania infantum** has been found in an obscure form of infantile splenomegaly in Algiers.

5. Genus Cercomonas.—(1) **Cercomonas hominis** has

been found in the feces in a variety of diarrheal conditions, and in from 10 to 25 per cent. of healthy persons in tropical regions. It is probably harmless. The body is 10 to 12 μ long, is pointed posteriorly, and has a flagellum at the anterior end (Fig. 114). The nucleus is difficult to make out.

Fig. 114.—Cercomonas hominis (about × 500): A, Larger variety; B, smaller variety
(Davaine).

6. Genus Bodo.—(1) **Bodo urinarius** is sometimes seen in the urine, darting about in various directions. It is probably an accidental contamination, or at most a harmless invader. It has a lancet-shaped body, about 10 μ long, and is somewhat twisted upon itself, with two flagella at the end.
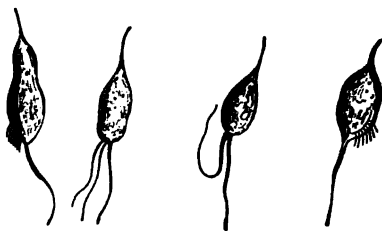
Fig. 115.—Trichomonas vaginalis (about × 1000) (after Kölliker and Scanzoni).

7. Genus Trichomonas.—(1) **Trichomonas Vaginalis.**—The acid discharge of catarrhal vaginitis sometimes contains this parasite in abundance. It is oval or pear-shaped, one to three times the diameter of a red blood-corpuscle in length, and has a cluster of flagella at one end (Fig. 115). As seen in fresh material it is not unlike

a pus-corpuscle in size and general appearance, but is
actively motile. When in motion the flagella are not
easily seen. No pathogenic significance is ascribed to
it in the vagina, but a few cases have been reported in
which it was apparently the cause of a urethritis in the
male. This and similar organisms, such as cercomonas
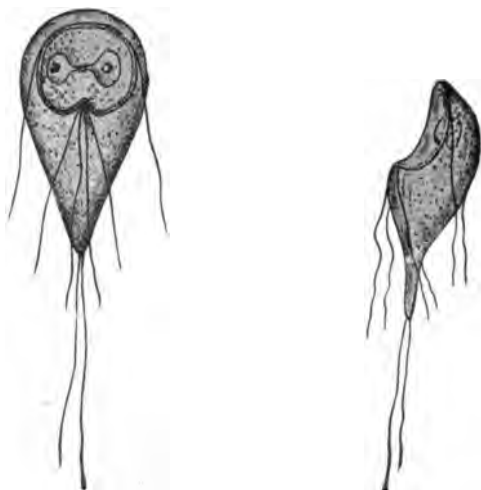
Fig. 116.—Lamblia intestinalis from the intestines of a mouse (about × 2000) (Grassi
and Schweiakoff).

and bodo, might be mistaken for spermatozoa by the
totally inexperienced worker.

(2) **Other Trichomonads.**—Various forms have been
described, regarded by some as identical with *T. vagi-
nalis*, by others as distinct species. Among these are
T. intestinalis, sometimes found in the feces in diarrheal
conditions, and *T. pulmonalis*, which has been encoun-
tered in the sputum of persons suffering from pulmonary
gangrene and putrid bronchitis.

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8. Genus Lamblia.—(1) **Lamblia intestinalis** is a very common parasite in the tropics, but is generally considered of little pathogenic importance. It is pear shaped, measures about 10 to 15 μ, and has a depression on one side of the blunt end, by which it attaches itself to the tops of the epithelial cells of the intestinal wall. Three pairs of flagella are arranged about the depression and one pair at the pointed end (Fig. 116).

SUBPHYLUM SPOROZOA

Class Telosporidia

All the members of this class are parasitic, but only a few have been observed in man, and only one genus, *Plasmodium*, is of much importance in human pathology. Propagation is by means of spores, and sporulation ends the life of the individual. In some species there is an alternation of generations, in one of which sexual processes appear. In such cases the male individual may be provided with flagella. Otherwise, there are no special organs of locomotion.

1. Genus Coccidium.—(1) **Coccidium cuniculi.**—This is a very common parasite of the rabbit and has been much studied; but extremely few authentic cases of infection in man have been reported. The parasite, which when fully developed is ovoid in shape and measures about 30 to 50 μ in length and has a shell-like integument, develops within the epithelial cells of the bile-passages. Upon reaching adult size it divides into a number of spores or merozoites which enter other epithelial cells and repeat the cycle. A sexual cycle outside the body, which suggests that of the malarial parasite, but does not require an insect host, also occurs.

Infection takes place from ingestion of the resulting sporozoites.

2. Genus Plasmodium.—This genus includes the malarial parasites which have already been described (p. 248).

3. Genus Babesia.—The proper position of this genus is uncertain. It is placed among the flagellates by some. The chief member is *Babesia bigeminum*, the cause of Texas fever in cattle. It is a minute, pear-shaped organism, lying in pairs within the red blood-corpuscles. An organism, *B.* (or *Piroplasma*) *hominis*, described as occurring in the red cells in "tick-fever" of Montana, is also placed in this genus, but its pathogenicity and even its existence are questionable.

SUBPHYLUM INFUSORIA
Class Ciliata

The conspicuous feature of this class is the presence of cilia. These are hair-like appendages which have a regular to-and-fro motion, instead of the irregular lashing motion of flagella. They are also shorter and more numerous than flagella. Most infusoria are of fixed shape and contain two nuclei. Contractile and food-vacuoles are also present. Encystment is common. Only one species is of medical interest. Certain ciliated structures, which have been described as infusoria, notably in sputum and nasal mucus, were probably ciliated body cells.

1. Genus Balantidium.—(1) **Balantidium Coli.**—This parasite, formerly called *Paramœcium coli*, is an occasional inhabitant of the colon of man, and sometimes produces diarrhea. It is an oval organism, about 0.1 mm. long, is covered with cilia, and contains a bean-shaped

macronucleus, a globular micronucleus, two contractile vacuoles, and variously sized granules (Fig. 117).

Fig. 117.—Balantidium coli (about × 300) (after Eichhorst).

Its ordinary habitat is the rectum of the domestic pig, where it apparently causes no disturbance. It probably reaches man in the encysted condition.

PHYLUM VERMIDEA

Of the worms, many species are parasitic in man and the higher animals. In some cases man is the regular host; in others, the usual habitat is some one of the animals, and the occurrence of the worm in man is more or less accidental. Such are called *incidental parasites.* Only those worms that are found in man with sufficient frequency to be of medical interest are mentioned here.

PHYLUM VERMIDEA

SUBPHYLUM I. **PLATYHELMINTHES.**—Flat-worms.

CLASS **Trematoda.**—Flukes. Unsegmented, leaf shaped.

Genus.	*Species.*
Fasciola.	F. hepatica.
Dicrocœlium.	D. lanceatum.
Opisthorchis.	Op. felineus.
	Op. sinensis.
Paragonimus.	P. westermani.
Schistosomum.	S. hæmatobium.
	S. japonicum.

CLASS **Cestoda.**—Tapeworms. Segmented, ribbon shaped.

Genus.	*Species.*
Tænia.	T. saginata.
	T. solium.
	T. echinococcus.
Hymenolepis.	H. nana.
Dipylidium.	D. caninum.
Dibothriocephalus.	D. latus.

SUBPHYLUM II. **NEMATHELMINTHES.**—Round-worms.

CLASS **Nematoda.**—Unsegmented, cylindric or fusiform.

Genus.	*Species.*
Anguillula.	A. aceti.
Ascaris.	A. lumbricoides.
Oxyuris.	O. vermicularis.
Filaria.	F. bancrofti.
	F. philippinensis.
	F. perstans.
	F. diurna.
	F. medinensis.
Uncinaria.	U. duodenalis.
Necator.	N. americanus.
Strongyloides.	S. intestinalis.
Trichinella.	T. spiralis.
Trichocephalus.	T. trichiuris.

SUBPHYLUM PLATYHELMINTHES
Class Trematoda

The trematode worms, commonly known as "flukes," are flat, unsegmented, generally tongue- or leaf-shaped worms. They are comparatively small, most species averaging between 5 and 15 mm. in length. They possess an incomplete digestive tract, without anus, and are provided with one or more sucking disks by means of which they can attach themselves to the host. Some are also provided with hooklets. Nearly all species are hermaphroditic, and the eggs of nearly all are operculated (provided with a lid), the only important exception

being *Schistosomum hematobium*, the egg of which has a characteristic spine. Development takes place by alternation of generations, the intermediate generation occurring in some water animal: mollusks, amphibians, fishes, etc.

1. Genus Fasciola.—(1) **Fasciola Hepatica.**—The " liver fluke " inhabits the bile-ducts of numerous herbivorous animals, especially sheep, where it is an important cause of disease. It brings about obstruction of the bile-passages, with enlargement and degeneration of the liver

Fig. 118.—Fasciola hepatica, about two-thirds natural size (Mosler and Peiper).

—" liver rot." A species of snail serves as intermediate host. The worm is leaf shaped, the average size being about 2.8 by 1.2 cm. The anterior end projects like a beak (head-cone 3 to 4 mm. long) (Fig. 118). Ova appear in the feces. They are yellowish brown, oval, operculated, and measure about 0.13 by 0.07 mm.

2. Genus Dicrocœlium.—(1) **Dicrocœlium lanceatum** is often associated with the liver fluke in the bile-passages of animals, but is neither so common nor so widely distributed geographically. It has rarely been observed in man. It is smaller (length about 1 cm.) and more

elongated. The long diameter of the eggs is about 0.04 mm.

3. Genus Opisthorchis.—(1) **Opisthorchis felineus** inhabits the gall-bladder and bile-ducts of the domestic cat and a few other animals. Infection in man has been repeatedly observed in Europe, and especially in Siberia. The body is flat, yellowish-red in color, and almost transparent. It measures 8 to 11 mm. by 1.5 to 2 mm. The eggs are oval, with a well-defined operculum at the narrower end, and contain a ciliated embryo when deposited. They measure about 30 by 11 μ.

(2) **Opisthorchis sinensis,** like the preceding fluke, inhabits the gall-bladder and bile-ducts of domestic cats and dogs. It is, however, much more frequent in man, being a common and important parasite in certain parts of Japan and China. The number present may be very great; over 4000 were counted in one case. The parasite resembles *Op. felineus* in shape and color. It is 10 to 14 mm. long and 2.5 to 4 mm. broad. The eggs have a sharply defined lid and measure 27 to 30 by 15 to 17 μ. When they appear in the feces they contain a ciliated embryo. The intermediate host is unknown.

4. Genus Paragonimus.—(1) **Paragonimus westermani,** called the "lung fluke," is also a common parasite of man in Japan, China, and Korea. It is likewise found in dogs, cats, and pigs in these countries, and, according to Ward and Stiles, in North America also. It inhabits the lung, causing the formation of small cavities. Moderate hemoptysis is the principal symptom. Ova are readily found in the sputum; the worms themselves are seldom seen, except *postmortem*. The worms are faint reddish-brown in color, egg shaped with the ventral

surface flattened, and measure 8 to 10 mm. by 4 to 6 mm. The ova, which are found in the sputum, are thin shelled, brownish yellow, and average about 0.093 by 0.057 mm. Little is known of the development outside the body.

5. Genus Schistosomum.—(1) **Schistosomum Hæmatobium.**—This trematode, frequently called *Bilharzia hæmatobia*, is an extremely common cause of disease (bilharziasis or Egyptian hematuria) in northern Africa, particularly in Egypt.

Unlike the other flukes, the sexes are separate. The male is 12 to 14 mm. long and 1 mm. broad. The body is flattened and the lateral edges curl ventrally, forming a longitudinal groove, in which the female lies (Fig. 119).

Fig. 119.—Schistosomum hæmatobium, male and female (about × 4) with eggs (about × 70) (von Jaksch).

The latter is cylindric in shape, about 20 mm. long and 0.25 mm. in diameter. The eggs are an elongated oval, about 0.15 mm. long, yellowish in color, and slightly transparent. They possess no lid, such as characterize the eggs of most of the trematodes, but are provided with a thorn-like spine which is placed at one end or laterally near the end.

In man the worm lives in the veins, particularly the portal vein and the veins of the bladder and rectum, leading to obstruction and inflammation. The eggs penetrate into the tissues and are present in abundance in the mucosa of the bladder and rectum. They also appear in the urine and feces. The mode of infection is unknown.

(2) **Schistosomum japonicum** resembles the preceding morphologically, but both the male and female are smaller. The ova present no spines and somewhat resemble those of *Uncinaria duodenalis*. It was discovered in Japan in 1904 and is apparently common in that country. It probably inhabits the arteries.

Class Cestoda

The cestodes, or tapeworms, are very common parasites of both man and the animals. In the adult stage they consist of a linear series of flat, rectangular segments (proglottides), at one end of which is a smaller segment, the scolex or head, especially adapted by means of sucking discs and hooklets for attachment to the host. The series represents a colony, of which the scolex is ancestor. The proglottides are sexually complete individuals (in most cases hermaphroditic), which are derived from the scolex by budding. With the exception of the immature segments near the scolex, each contains a uterus filled with ova.

The large tapeworms, *Tænia saginata*, *T. solium*, and *Dibothriocephalus latus*, are distinguished from one another mainly by the structure of the scolex and the uterus. The scolex should be studied with a low-power objective or a hand lens. The uterus is best seen by pressing the segment out between two plates of glass.

All the tapeworms pass a larval stage in the tissues of an intermediate host, which is rarely of the same species as that which harbors the adult worm. From the ova which have developed in the proglottides of the adult worm, and which pass out with the feces of the host,

there develop embryos, or *oncospheres*, each provided with three pairs of horny hooklets. When the oncosphere is taken into the intestines of a suitable animal, it penetrates to the muscles or viscera and there forms a cyst in which develop usually one, but sometimes many, scolices, which are identical with the head of the adult worm. When the flesh containing this cystic stage is eaten without sufficient cooking to destroy the scolices, the latter attach themselves to the intestinal wall and produce adult tapeworms by budding.
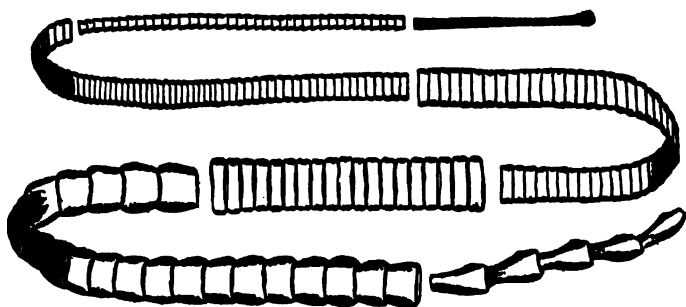
Fig. 120.—Tænia saginata (Eichhorst).

Ordinarily, only the adult stage occurs in man. In the case of *Tænia echinococcus* only the larval stage is found. *T. saginata* and *T. solium* may infect man in either stage, although the cystic stage is very rare.

Since the head, or scolex, is the ancestor from which the worm is formed in the intestine, it is important, after giving a vermifuge, to make certain that the head has been passed with the worm. Should it remain, a new worm will develop.

The principal tapeworms found in man belong to the genera Tænia, Hymenolepis, and Dibothriocephalus.

1. Genus Tænia.—(1) **Tænia Saginata or T. Medio-canellata** (Fig. 120).—This, the beef tapeworm, is the common tapeworm of the United States. Its length sometimes exceeds twenty-five feet. The middle segments measure about 6 by 15 mm. The scolex is about the size of a pin-head, and is surrounded by four sucking discs, but has no hooklets (Fig. 122). The uterus extends along the midline of the segment and gives off about twenty branches upon each side (Fig. 129).
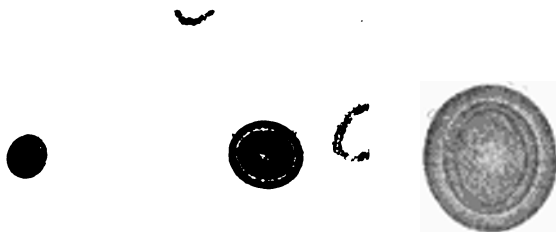
Fig. 121.—Eggs of Tænia saginata, magnifications 100, 250, and 500 diameters (photographs by the author).

The larval stage is passed in the muscles of various animals, especially cattle.

The scolex is ingested with the meat, its capsule is dissolved by the digestive juices, and it attaches itself to the intestinal wall by means of its suckers. It then develops into the mature worm.

The ova are present in the stools of infected persons, often in great numbers. They are spheric or ovoid, yellow in color, and have a thick, radially striated shell (Fig. 121). Their greatest diameter is 30 to 40 μ (about four or five times the diameter of a red blood-corpuscle).

Vegetable cells, which are generally present in the feces, are often mistaken for them.

(2) **Tænia solium,** the pork tapeworm, is very rare in this country. It is usually much shorter than *Tænia saginata.* The scolex is surrounded by four sucking discs, and has a projection, or rostellum, with a double row of horny hooklets (Fig. 123). The uterus has only seven to ten branches (Fig. 129).

Fig. 122.—Head of Tænia saginata (Mosler and Peiper).

Fig. 123.—Head of Tænia solium (Mosler and Peiper).

The cysticercus stage occurs ordinarily in the muscles of the pig, but is occasionally seen in man, most frequently affecting the brain and eye (*Cysticercus cellulosæ*).

The ova closely resemble those of *Tænia saginata,* but are a little smaller (Fig. 130).

(3) **Tænia Echinococcus.**—The mature form of this tapeworm inhabits the intestines of the dog and wolf. The larvæ develop in cattle and sheep ordinarily, but are sometimes found in man, where they give rise to echinococcus or "hydatid" disease. The condition is unusual

in America, but is not infrequent in Central Europe and is common in Iceland and Australia.

The adult parasite is 2.5 to 5 mm. long and consists of only four segments (Fig. 124). It contains many ova. When the ova reach the digestive tract of man the embryos are set free and find their way to the liver, lung, or other organ, where they develop into cysts, thus losing their identity. The cysts may attain the size of a child's head. Other cysts, called " daughter-cysts," are formed within these. The cyst-wall is made up of two layers, from the inner of which develop larvæ which are identical with the head, or scolex, of the mature parasite. These are ovoid structures 0.2 to 0.3 mm. long. Each has four lateral suckers

Fig. 124.—Tænia echinococcus; enlarged (Mosler and Peiper).

and a rostellum surmounted by a double circular row of horny hooklets. The rostellum with its hooklets is frequently invaginated into the body.

Diagnosis of echinococcus disease depends upon detection of scolices, free hooklets which have fallen off from degenerated scolices, or particles of cyst-wall, which is characteristically laminated and usually has curled edges. The lamination is best seen at the torn edge of the membrane. These can be found in fluid withdrawn from the cysts or, less frequently, in the sputum or the urine, when the disease involves the lung or kidney (Figs. 59 and 125). The cysts are sometimes "barren," growing to a considerable size without producing scolices.

The cyst fluid is clear, between 1.009 and 1.015 in

specific gravity, and contains a notable amount of sodium chlorid, but no albumin.

2. Genus Hymenolepis.—(1) **Hymenolepis nana,** the dwarf tapeworm (Fig. 126), is 1 to 1.5 cm. in length and 0.5 to 0.7 mm. in breadth at the widest part. The head is globular and has a rostellum with a crown of 24
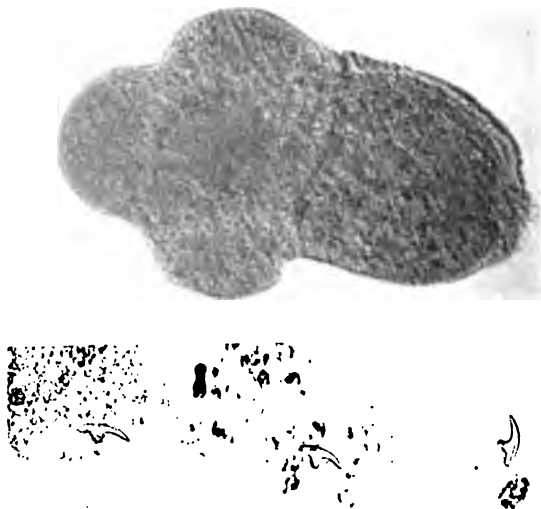
Fig. 125.—Scolex and hooklets of Tænia echinococcus in fluid from hepatic cyst (× 300) (photographs by the author).

to 30 hooklets. There are about 150 segments The eggs are round or oval, 30 to 40 μ in diameter, and resemble those of *Tænia saginata*. The worm is common in Europe and America. It is most frequent in children and is generally present in large numbers, producing considerable digestive and nervous disturbances. The mode of infection is unknown.

3. **Genus Dipylidium.**—(1) **Dipylidium caninum,** sometimes called *Tænia elliptica*, is a very common tape-worm of dogs and cats. It is about 20 cm. long and 2 to 3 mm. broad. The intermediate host is the flea or louse. Infection of human beings is not common, and is mostly confined to children, who are probably infected from the dog licking their mouths or from getting lice or fleas into their mouths.

Fig. 126.—Hymen-olepis nana, about natural size (Mosler and Peiper).

4. **Genus Dibothriocephalus.**—(1) **Dibothriocephalus latus,** the fish tape-worm, sometimes reaches fifty feet in length, although it is generally not more than half so long. When several worms are present, they are much smaller. It is common in some countries of Europe, especially Ireland, and in Japan, but is very rare in this country.

Fig. 127.—Head of Dibothriocephalus latus (about ×9): *a, a,* Head grooves; *b,* neck (Blanchard).

The head is about 1 mm. broad and is not unlike the bowl of a spoon in shape. It is unprovided with either suckers or hooklets, but has two longitudinal grooves which serve the same purpose (Fig. 127). The length of the segments is generally less than their breadth, mature segments measuring about 3 by 10 or 12 mm. The uterus, which is situated in the center of the segment, is roset shaped (Fig. 129) and brown or black in color.

The larval stage is found in fish, especially the pike.
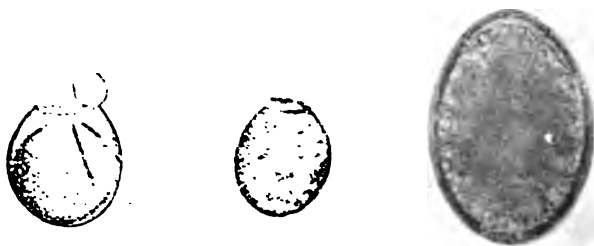
Fig. 128.—Ova of Dibothriocephalus latus ($\times$ 250 and 500). The lids were forced open by pressure upon the cover-glass (photographs by the author).

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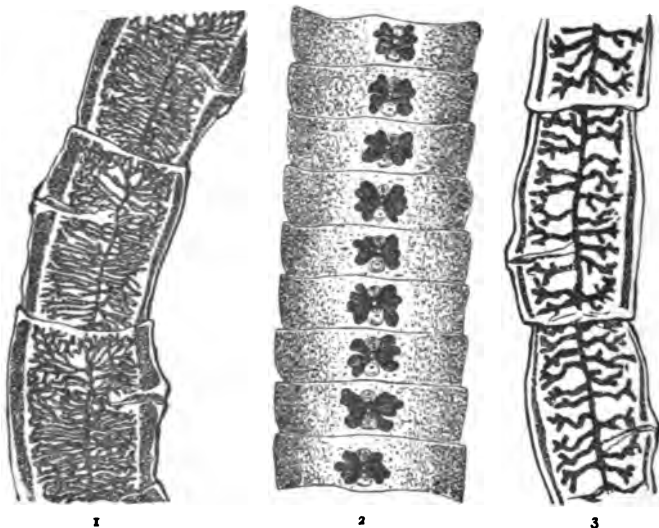
Fig. 129.—Segments of—(1) Tænia saginata; (2) Dibothriocephalus latus; (3) Tænia solium, showing arrangement of uterus.

The ova are characteristic. They measure about 45 by 70 μ, are brown in color, and are filled with small

spherules. The shell is thin and has a small hinged lid at one end. As the eggs appear in the feces the lid is not easily seen, but it may be demonstrated by sufficient pressure upon the cover-glass to force it open (Fig. 128). The only other operculated eggs met with in man are those of the fluke-worms.
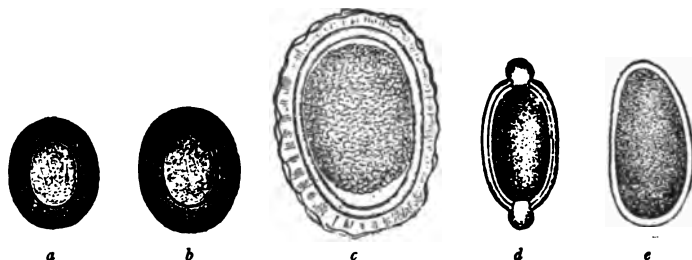
Fig. 130.—Comparative size of eggs of intestinal parasites (about × 400): *a*, Tænia solium; *b*, Tænia saginata; *c*, Ascaris lumbricoides; *d*, Trichocephalus trichiurus; *e*, Oxyuris vermicularis (after Strümpell).

Dibothriocephalus latus is interesting clinically because it often causes a very severe grade of anemia, which may be indistinguishable from pernicious anemia.

SUBPHYLUM NEMATHELMINTHES

Class Nematoda

The nematodes, or round-worms, are cylindric or fusiform worms, varying in length, according to species, from 1 mm. to 40 or 80 cm. As a rule, the sexes are separate. The male is smaller and more slender than the female. In a few cases the female is viviparous; in most cases she deposits ova which are characteristic, so that the finding of a single egg may establish the diagnosis. Except in a few instances the young are different from the adult, and must pass a certain larval stage of development before again reaching a host.

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An intermediate host is, however, necessary with only a few species.

1. Genus Anguillula.—(1) **Anguillula Aceti.**—This worm, commonly called the "vinegar eel," is usually present in vinegar. A drop of the vinegar, particularly of the sediment, will frequently show great numbers, all in active motion: males, about 1 or 1.5 mm. long; females, somewhat larger and frequently containing several coiled embryos; and young, of all sizes up to the adult (Fig. 60).

The vinegar eel is never parasitic, but is occasionally met with as a contamination in the urine (see p. 171), and has there been mistaken for the larva of filaria or strongyloides.

2. Genus Ascaris.—(1) **Ascaris Lumbricoides.**—The female is 20 to 40 cm. long and about 6 mm. thick (Fig. 131); the male, a little more than half as large. Their color is reddish or brown. They are the common "round-worms" so

Fig. 131.—Ascaris lumbricoides (female) (Mosler and Peiper).

frequently found in children. Their habitat is the small intestine. Large numbers are sometimes present.

The diagnosis is made by detection of the worms or ova in the feces. The latter are generally numerous. They

are elliptic, measuring about 50 by 70 μ, and have an unsegmented protoplasm (Fig. 132). The shell is thick and is surrounded by an uneven gelatinous envelop which is often stained with bile.

The eggs do not hatch in the intestine of the original host. They pass out in the feces and, after a variable period, usually about five weeks, come to contain an embryo which remains within the shell until ingested by a new host. The embryo is very resistant and may
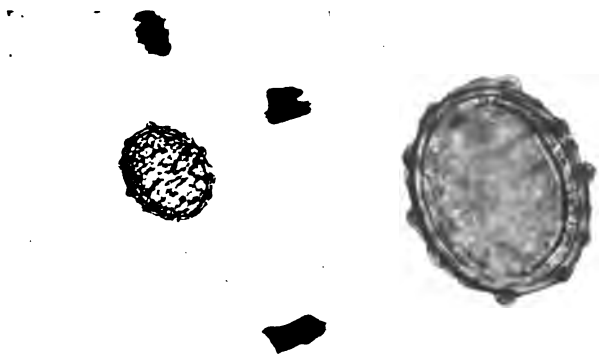
Fig. 132.—Ova of Ascaris lumbricoides (×250 and 500) (photographs by the author).

remain alive within the shell for years. Upon reaching the intestine of the new host it hatches out and develops into the adult worm.

3. Genus Oxyuris.—(1) **Oxyuris Vermicularis.**—This is the "thread-worm" or "pin-worm" which inhabits the colon and rectum, especially of young children. Its presence should be suspected in all unexplained cases of pruritus ani. The female is about 1 cm. long; the male, about 0.6 cm. (Fig. 133).

The worms are not infrequently found in the feces; the ova, rarely. The latter are best found by scraping the skin at the margin of the anus, where they are deposited by the female, who wanders out from the rectum for this purpose, this producing the troublesome itching. They are asymmetrically oval with one flattened side, are about 50 μ in length, and often contain a partially developed embryo. The diagnosis is best made by giving a purgative and searching the stool for the adult worms.

Infection takes place through swallowing the ova. Auto-infection is likely to occur in children; the ova cling to the fingers after scratching and are thus carried to the mouth.
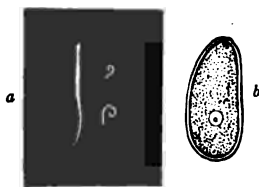
Fig. 133.—Oxyuris vermicularis and egg: *a*, Male and female, natural size; *b*, egg (about × 250) (after Heller).

4. Genus Filaria.—(1) **Filaria Bancrofti.**—The adults are thread-like worms, the male about 4 cm., the female about 8 cm., long. They live in pairs in the lymphatic channels and glands, especially those of the pelvis and groin, and often occur in such numbers as to obstruct the flow of lymph. This is the most common cause of elephantiasis. Infection is very common in tropical countries, especially in Samoa, the West Indies, Central America, and the Isthmus of Panama. It is said that in Samoa 50 per cent. of the natives are infected.

The female is viviparous, and produces vast numbers

of embryos, which appear in the circulating blood. The name *Filaria sanguinis hominis*, which is commonly applied to them, is incorrect, since they do not constitute a species. These embryos are about as wide as a red corpuscle and 0.2 to 0.4 mm. long (Fig. 99), and are very actively motile. They are found in the peripheral blood only at night, appearing about 8 P. M., and reaching their maximum number—which is
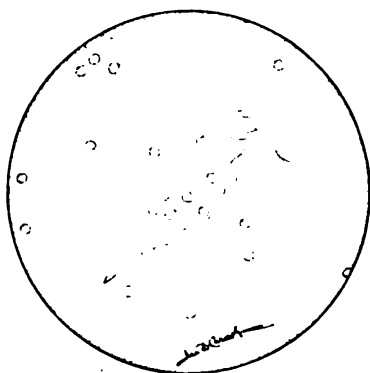
Fig. 134.—Embryo of Filaria bancrofti in chylous hydrocele fluid; length, 300 μ; width, 8 μ. A number of red blood-corpuscles also appear (studied through courtesy of Dr. S. D. Van Meter).

sometimes enormous—about midnight. If the patient change his time of sleeping, they will appear during the day. Infection is carried by a variety of mosquito, which acts as intermediate host. Diagnosis rests upon detection of embryos in the blood, as described on p. 256.

The embryos are sometimes found in urine and chylous fluids from the serous cavities. Their motion is then usually less active than when in blood. That shown in Fig. 134 was alive sixty hours after removal of the

fluid. Embryos were present in the blood of the same patient.

A number of other filariæ whose larvæ appear in the blood are known, some of them only in the larval stage. Among these are *Filaria philippinensis* and *F. perstans*, which exhibit no periodicity, and *F. diurna* and *F. loa*, whose embryos appear in the blood during the day. The adult of the last named is especially frequent in the orbit and beneath the conjunctiva.

(2) **Filaria medinensis,** the "guinea-worm," is a very interesting and important worm of Africa and southern Asia. It is thought to be the "fiery serpent" which molested the Children of Israel in the Wilderness.

The larva probably enters the body through the skin or gastro-intestinal tract. It wanders about in the subcutaneous tissues until maturity, producing slight, if any, symptoms. The male has only recently been discovered. It is only 4 cm. long. It dies soon after the female is impregnated. The adult female is a very slender, yellowish worm, about 50 to 80 cm. long, its appearance somewhat suggesting a catgut suture. When gestation is complete the greater part of the female's body consists of a uterus filled with embryos. The female then travels to the feet or ankles of the host and there produces a red nodule and, finally, an ulcer, from the center of which her head protrudes. Through this great numbers of embryos are discharged whenever it comes in contact with water. Little damage is done unless the worm is pulled out, when the embryos are set free in the tissues and cause serious disturbances.

When discharged the embryos seek out a small crustacean, cyclops, which serves as intermediate host.

5. Uncinaria Duodenalis and Necator Americanus.

—These, the Old and the New World hook-worm respectively, are among the more harmful of the animal parasites. They inhabit the small intestine, often in great numbers, and commonly produce a severe and often fatal anemia. The presence of a few, however, may cause slight, if any, disturbance.
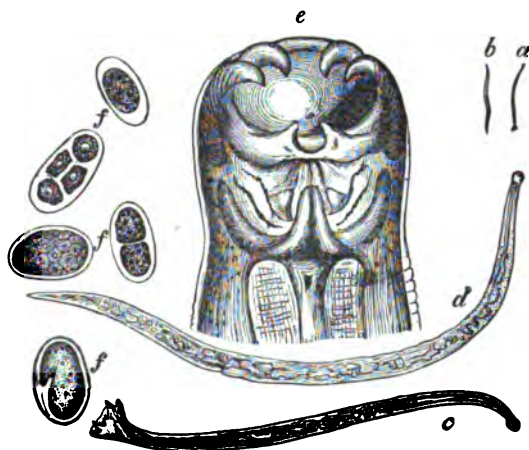
Fig. 135.—Uncinaria duodenalis: *a*, Male (natural size); *b*, female (natural size); *c*, male (enlarged); *d*, female (enlarged); *e*, head; *f*, *f*, *f*, eggs (after v. Jaksch).

Uncinaria duodenalis is common in southern Europe and in Egypt. The body is cylindric, reddish in color, and the head is bent sharply. The oral cavity has six hook-like teeth. The female is 12 to 18 mm. long and the tail is pointed. The male is 8 to 10 mm. long and the posterior end is expanded into an umbrella-like pouch, the caudal bursa. The eggs are oval and have a thin, smooth, transparent shell. As they appear in the feces the protoplasm is divided into 2, 4, 8, or more rounded

segments (Fig. 135). They measure 32 to 40 μ by 55 to 65 μ.

Necator americanus is very common in subtropical America, including the southern part of the United States and the West Indies. In Porto Rico 90 per cent. of the rural population is infected. Isolated cases, probably imported, have been seen in most of the Northern
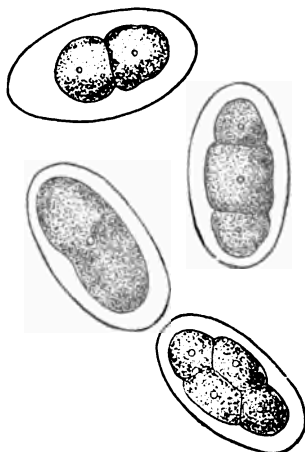
Fig. 136.—Four eggs of the New World hook-worm (Necator americanus), in the one-, two-, and four-cell stages. The egg showing three cells is a lateral view of a four-cell stage (about ×350) (after Stiles).

States. The American hook-worm is smaller than the Old World variety, the male being 7 to 9 mm. long, the female 9 to 11 mm. The four ventral hook-like teeth are replaced by chitinous plates. There are also differences in the caudal bursa of the male, and in the situation of the vulva in the female. The ova (Fig. 136) resemble those of *Uncinaria duodenalis*, but are larger, 36 to 40 μ by 67 to 75 μ.

The life-history of the two worms is probably the same.

The ova pass out with the feces, and, under favorable conditions of warmth and moisture, develop an embryo which hatches within a few days. The resulting larvæ pass through a stage of development in warm moist earth, growing to a length of 0.5 to 0.6 mm., and moulting twice. They are then ready to infect a new host. In some cases they probably reach the host's intestine by way of the mouth, with food or water; but the usual route is probably that established by Loos. When moist earth containing the larvæ comes in contact with the skin, they penetrate into the subcutaneous tissues. This is favored by retention of mud between the toes of those who go barefooted. When the larvæ are abundant a dermatitis is induced ("ground itch"). From the subcutaneous tissue they pass by way of lymph- and bloodstreams to the lungs. Here they make their way into the smaller bronchi, are carried by the bronchial mucus to the pharynx, and are swallowed. They thus ultimately reach the small intestine, where they develop into mature worms.

The diagnosis of hook-worm infection, which is assuming increasing importance in this country, must rest upon detection of ova in the feces. The worms themselves seldom appear except after thymol and a cathartic. A small portion of the feces, diluted with water if necessary, is placed upon a slide, covered, and searched with a 16 mm. objective. A higher power may rarely be necessary to positively identify an egg, but should not be used as a finder. The eggs are nearly always typic, showing a thin but very distinct shell, a clear zone, and a segmented protoplasm, and after having once been seen are not easily mistaken. In severe infections eggs may

be found in every microscopic field; in most cases, even though comparatively mild, they can be found on the first slide examined. It is seldom necessary to search more than half a dozen slides. When they are scarce, some method of sedimenting the feces may be tried, but this is rarely necessary.

6. Genus Strongyloides.—(1) **Strongyloides Intestinalis.**—Infection with this worm is by no means so rare in this country as the few clinical reports would indicate. It is very common in subtropical countries, notably in Italy and in southern China. It seems probable that the parasite is the cause of " Cochin China diarrhea," although some authorities regard it as harmless.

The adult worm, which reproduces by parthenogenesis, is about 2 mm. long. It inhabits the upper portion of the small intestine, but neither it nor the ova appear in the stool unless an active diarrhea exists. Ordinarily the eggs hatch in the intestines, and when infection is severe embryos can be found in the feces in large numbers. These are the "rhabditiform embryos," which measure about 0.40 by 0.02 mm. They are actively motile, and are best found by making a small depression in the fecal mass, filling it with water, and keeping in a warm place (preferably an incubator) for twelve to twenty-four hours. The embryos will collect in the water, and can be easily found by transferring a drop to a slide and examining with a 16 mm. objective. The inexperienced worker should make sure that the worms *move*, or he may be misled by the vegetable spines which are generally present in the feces. Certain of these spines (notably those from the skin of a peach) closely resemble small worms.

Outside the body the rhabditiform embryos develop into a free-living, sexually differentiated generation. The young of this generation are the more slender "filariform embryos" (Fig. 137). Infection can occur either through these embryos of the free-living generation or by direct transformation of rhabditiform into filariform embryos, and these into the parthenogenic parasitic adult.

Fig. 137.—Strongyloides intestinalis: A, Mature female; B, rhabditiform larva; C, filariform larva (after Braun).

7. Genus Trichinella.—(1) **Trichinella Spiralis.**—This is a very small worm, not exceeding 3 mm. in length when fully developed. Infection in man occurs from ingestion of insufficiently cooked pork, which contains encysted embryos. Ordinary "curing" of pork does not kill them. These reach maturity in the small intestine. Soon after copulation the males die, and the females penetrate into the mucous membrane. They live in this situation about six weeks, giving birth to great numbers of young, averaging as high as 1500 from a single female. The larvæ migrate to the striated muscles, chiefly near the tendinous insertions, where they grow to a length of about 0.8 mm., and finally become encysted. In this condition they may remain alive and capable of developing for as long as twenty-five years.

Trichiniasis is generally accompanied by a marked eosinophilia. The diagnosis is made by teasing out upon a slide a bit of muscle, obtained preferably from the outer head of the gastrocnemius, the insertion of the deltoid,

Fig. 138.—Trichinella spiralis (larvæ) from head of right gastrocnemius muscle; seventh week of disease (two-thirds objective; eye-piece 4) (Boston).

or the lower portion of the biceps. The coiled embryos can easily be seen with a 16 mm. objective (Fig. 138). The embryos can be found in the blood (p. 257) before they have reached their final resting-place in the muscles.

Fig. 139.—Trichocephalus trichiurus: a, Female; b, male (natural size) (Heller).

8. Genus Trichocephalus.—(1) **Trichocephalus Trichiurus.**—This, the " whip-worm," is 4 or 5 cm. long. Its anterior portion is slender and thread-like, while the posterior portion is thicker (Fig. 139). It is

widely distributed geographically, and is one of the most common of intestinal parasites in this country. It lives in the large intestine, especially the cecum, with its slender extremity embedded in the mucous membrane. Whip-worms do not, as a rule, produce any symptoms, although gastro-intestinal disturbances, nervous symptoms, and anemia have been ascribed to them. They, as well as many other intestinal parasites, are probably
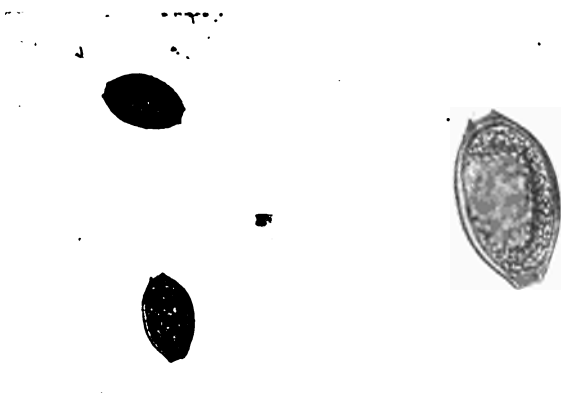
Fig. 140.—Ova of Trichocephalus trichiurus (× 250 and 500) (photographs by the author).

an important factor in the etiology of appendicitis, typhoid fever, and other intestinal infections. The damage which they do to the mucous membrane favors bacterial invasion.

The number present is usually small. The worms themselves are rarely found in the feces. The ova, which are not often abundant, are easily recognized. They are brown, ovoid in shape, about 50 μ long, and have a button-like projection at each end (Fig. 140).

PHYLUM ARTHROPODA

The arthropoda which are parasitic to man belong to the classes Arachnoidea and Insecta. They are nearly all external parasites, and the reader is referred to the standard works upon diseases of the skin for descriptions. The several species of the louse (*Pediculus capitis, P. vestimenti, P. pubis*), the itch mite (*Sarcoptes scabiei*), and the small organism (*Demodex folliculorum*) which lives in the sebaceous glands, especially about the face, are the most common members of this group.

A number of flies may deposit their ova in wounds or in such of the body cavities as they can reach, and the resulting maggots may cause intense irritation. Ova may be swallowed with the food and the maggots appear in the feces. Probably most important is the "screw worm," the larva of *Chrysomyia macellaria*, infection with which is not rare in some parts of the United States. The ova are most commonly deposited in the nasal passages, and the larvæ, which may be present in great numbers, burrow through the soft parts, cartilage, and even bone, always with serious and often with fatal results.

CHAPTER VII

MISCELLANEOUS EXAMINATIONS

PUS

PUS contains much granular débris and numerous more or less degenerated cells, the great majority being polymorphonuclear leukocytes—so-called "pus-corpuscles." Eosinophilic leukocytes are common in gonorrheal pus and in asthmatic sputum. Examination of pus is directed chiefly to detection of bacteria.

When very few bacteria are present, culture methods, which are outlined in Chapter VIII, must be resorted to. When considerable numbers are present, they can be detected and often identified in cover-glass smears. Several smears should be made, dried, and fixed, as described on p. 407.

One of these should be stained with a bacterial stain. Löffler's methylene-blue and Pappenheim's pyronin-methyl-green are especially satisfactory for this purpose. These stains are applied for one-half minute to two minutes or longer, without heating; the preparation is rinsed in water, dried, mounted, and examined with an oil-immersion lens. Another smear should be stained by Gram's method. These will give information concerning all bacteria which may be present, and frequently no other procedure will be necessary for their identification.

The most common pus-producing organisms are *staphylococci* and *streptococci*. They are both cocci, or spheres, their average diameter being about 1 μ. Staphylococci are commonly grouped in clusters, often compared to bunches of grapes (Fig. 141). There are several varieties which can be distinguished only in cultures. Streptococci are arranged side by side, forming chains of variable length (Fig. 142). Sometimes there are only three or four individuals in a chain; sometimes a chain
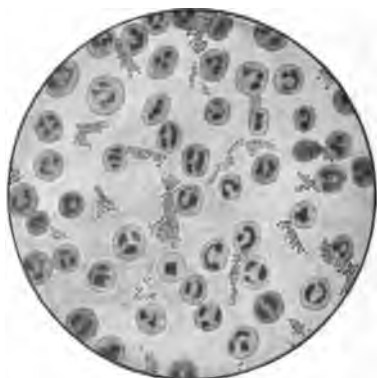
Fig. 141.—Staphylococcus pyogenes albus from an abscess of the parotid gland (Jakob).

is so long as to extend across several microscopic fields. Streptococci are more virulent than staphylococci, and are less commonly met. Both are Gram-positive. Their cultural characteristics are given on p. 415.

Should bacteria resembling *pneumococci* be found, Buerger's or Smith's method for capsules (p. 55) should be tried. When these are not available, capsules can usually be shown by the method of Hiss. The dried and fixed smear is covered with a stain composed of 5 c.c. saturated alcoholic solution gentian-violet and 95 c.c.

distilled water, and heated until steam rises. The preparation is then washed with 20 per cent. solution of copper sulphate, dried, and mounted in Canada balsam.

Pneumococci may give rise to inflammation in many locations (see p. 54). When they form short chains, demonstration of the capsule is necessary to distinguish them from streptococci.

If tuberculosis be suspected, the smears should be stained by one of the methods for the *tubercle bacillus*
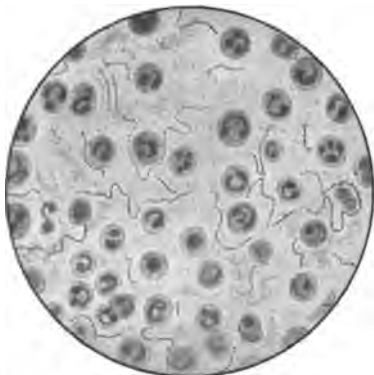
Fig. 142.—Streptococcus pyogenes from a case of empyema (Jakob).

(pp. 49 and 51), or guinea-pigs may be inoculated. The bacilli are generally difficult to find in pus, and bacteria-free pus would suggest tuberculosis.

Gonococci, when typic, can usually be identified with sufficient certainty for clinical purposes in the smear stained with Löffler's methylene-blue or, much better, Pappenheim's pyronin-methyl-green. They are coffeebean-shaped cocci which lie in pairs with their flat surfaces together (Fig. 144). They lie for the most part within pus-cells, an occasional cell being filled with them,

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while the surrounding cells contain few or none. A few
are found outside of the cells. It is not usual to find
gonococci when many other bacteria are present, even

Fig. 143.—Diplococcus pneumoniæ from ulcer of cornea (obj. one-twelfth oil immersion)
(study through courtesy of Dr. C. A. Oliver) (Boston).

though the pus is primarily of gonorrheal origin. When-
ever the identity of the organism is at all questionable,
Gram's method should be tried. In rare instances it

Fig. 144.—Gonococci in urethral pus (McFarland).

may be necessary to resort to cultures. The gonococcus
is distinguished by its failure to grow upon ordinary
media (see p. 416).

Gonococci are generally easily found in pus from untreated acute and subacute gonorrheal inflammations—conjunctivitis, urethritis, etc.—but are found with difficulty in pus from chronic inflammations and abscesses, and in urinary sediments.

PERITONEAL, PLEURAL, AND PERICARDIAL FLUIDS

The serous cavities contain very little fluid normally, but considerable quantities are frequently present as a result of pathologic conditions. The pathologic fluids are classed as transudates and exudates.

Transudates are non-inflammatory in origin. They contain only a few cells, and less than 2.5 per cent. of albumin, and do not coagulate spontaneously. The specific gravity is below 1.018. Micro-organisms are seldom present.

Exudates are of inflammatory origin. They are richer in cells and albumin, and tend to coagulate upon standing. The specific gravity is above 1.018. Bacteria are generally present, and often numerous. The amount of albumin is estimated by Esbach's method, after diluting the fluid. Bacteria are recognized by cultures, animal inoculation, or stained smears.

Exudates are usually classed as serous, serofibrinous, seropurulent, purulent, putrid, and hemorrhagic, which terms require no explanation. In addition, chylous and chyloid exudates are occasionally met, particularly in the peritoneal cavity. In the chylous form the milkiness is due mainly to the presence of minute fat-droplets, and is the result of rupture of a lymph-vessel usually from obstruction of the thoracic duct. Chyloid exudates are milky chiefly from proteins in suspension, or fine

débris from broken-down cells. These exudates are most frequently seen in carcinoma and tuberculosis of the peritoneum.

Cytodiagnosis.—This is diagnosis from a differential count of the cells in a transudate or exudate, particularly one of pleural or peritoneal origin.

The fresh fluid, obtained by aspiration, is centrifugalized for at least five minutes; the supernatant liquid is

Fig. 145.—Cytodiagnosis. Polymorphonuclear leukocytes and swollen endothelial cells from acute infectious non-tuberculous pleuritis (Percy Musgrave; photo by L. S. Brown).

poured off; and cover-glass smears are made and dried in the air. The smears are then stained with Wright's blood-stain, to which one-third its volume of pure methyl-alcohol has been added. Cover the smear with this fluid for one-half minute, then dilute with 8 or 10 drops of water, and let stand about two minutes. Wash gently in water, and dry by holding the cover-glass between the fingers over a flame. Mount in balsam and examine with an oil-immersion objective.
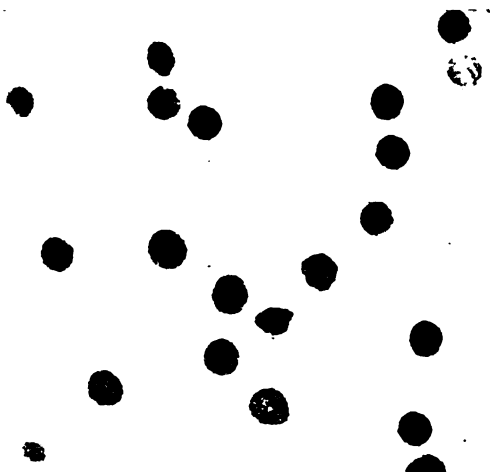
Fig. 146.—Cytodiagnosis. Lymphoid cells from pleural fluid; case of tuberculous pleuritis
(Percy Musgrave; photo by L. S. Brown).
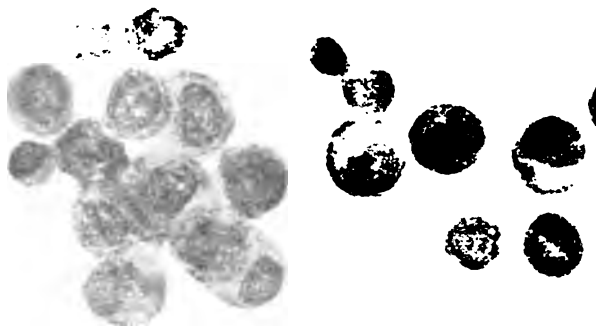
Fig. 147.—Cytodiagnosis. Endothelial cells from transudate or mechanical effusion
(Percy Musgrave; photo by L. S. Brown).

Predominance of polymorphonuclear leukocytes (pus-corpuscles) points to an acute infectious process (Fig. 145).

Predominance of lymphocytes (Fig. 146) generally signifies tuberculosis. Tuberculous pleurisy due to direct extension from the lung may give excess of polymorphonuclears owing to mixed infection.

Predominance of endothelial cells, few cells of any kind being present, indicates a transudate (Fig. 147). Endothelial cells generally predominate in carcinoma, but are accompanied by considerable numbers of lymphocytes and red blood-corpuscles.

CEREBROSPINAL FLUID

Examination of the fluid obtained by lumbar puncture is of value in diagnosis of certain forms of meningitis.

Tubercle bacilli can be found in the majority of cases of tuberculous meningitis. The sediment, obtained by thorough centrifugalization or by coagulation and treatment with antiformin, is spread upon slides and stained by one of the methods already given. A considerable number of smears should be examined. In doubtful cases inoculation of guinea-pigs must be resorted to.

The *Diplococcus intracellularis meningitidis* is recognized as the cause of epidemic cerebrospinal fever, and can be detected in the cerebrospinal fluid of most cases, especially those which run an acute course. Cover-glass smears from the sediment should be stained by the method for the gonococcus (p. 369). The meningococcus is an intracellular diplococcus which often cannot be distinguished from the gonococcus in stained smears (Fig. 148). It, also, decolorizes by Gram's method. The presence of such a diplococcus in meningeal exudates is, however, sufficient for its identification.

Various organisms have been found in other forms of meningitis—the pneumococcus most frequently. In

Fig. 148.—Diplococcus intracellularis meningitidis in leukocytes (× 2000) (Wright and Brown).

some cases no micro-organisms can be detected even by culture methods.

ANIMAL INOCULATION

Inoculation of animals is one of the most reliable means of verifying the presence of certain micro-organisms in fluids and other pathologic material, and is helpful in determining the species of bacteria which have been isolated in pure culture.

Clinically, it is applied almost exclusively to demonstration of the tubercle bacillus when other means have failed or are uncertain. The guinea-pig is the most

suitable animal for this purpose. When the suspected material is fluid and contains pus, it should be well centrifugalized, and one or two cubic centimeters of the sediment injected by means of a large hypodermic needle into the peritoneal cavity or underneath the loose skin of the groin. Fluids from which no sediment can be obtained must be injected directly into the peritoneal cavity, since at least 10 c.c. are required, which is too great an
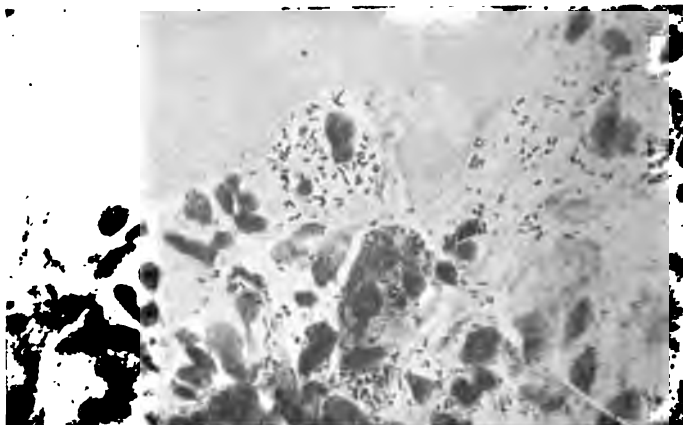
Fig. 149.—Influenza bacilli in spinal fluid. Case of meningitis ($\times$ 1000) (photograph by the author).

amount to inject hypodermically. Solid material should be placed in a pocket made by snipping the skin of the groin with scissors, and freeing it from the underlying tissues for a short distance around the opening. When the intraperitoneal method is selected, several animals must be inoculated, since some are likely to die from peritonitis caused by other organisms before the tubercle bacillus has had time to produce its characteristic lesions.

The animals should be killed at the end of six or eight

weeks, if they do not die before that time, and a careful
postmortem examination should be made for the char-
acteristic pearl-gray or yellow tubercles scattered over
the peritoneum and through the abdominal organs, par-
ticularly the spleen, and for caseous inguinal and retro-
peritoneal lymph-glands.   The tubercles and portions of
the caseous glands should be crushed between two slides,
dried, and stained for tubercle bacilli.   The bacilli are
difficult to find in the caseous material.

THE MOUTH

Micro-organisms are always present in large numbers.
Among these is *Leptothrix buccalis* (Fig. 150), which is

Fig. 150.—Gingival deposit (unstained): *a*, Squamous epithelial cells; *b*, leukocytes; *c*,
bacteria; *d*, Leptothrix buccalis (Jakob).

especially abundant in the crypts of the tonsils and the
tartar of the teeth.   The whitish patches of *Pharyn-
gomycosis leptothrica* are largely composed of these fungi.
They are slender, segmented threads, which generally,
but not always, stain violet with Lugol's solution, and are

readily seen with a 4 mm. objective. At times they are observed in the sputum and stomach fluid. In the former they might be mistaken for elastic fibers; in the latter, for Boas-Oppler bacilli. In either case, the reaction with iodin will distinguish them.

Thrush is a disease of the mouth seen most often in children, and characterized by the presence of white patches upon the mucous membrane. It is caused by the thrush fungus, *Oïdium albicans.* When a bit from one

Fig. 151.—Thrush fungus (Oïdium albicans) (Jakob).

of the patches is pressed out between a slide and cover and examined with a 4 mm. objective, the fungus is seen to consist of a network of branching segmented hyphæ with numerous spores, both within the hyphæ and in the meshes between them (Fig. 151). The meshes also contain leukocytes, epithelial cells, and granular débris.

Acute pseudomembranous inflammations, which occur chiefly upon the tonsils and nasopharynx, are generally caused by the diphtheria bacillus, but may result from

streptococcic infection. In many cases diphtheria bacilli can be demonstrated in smears made from the membrane and stained with Löffler's methylene-blue or 2 per cent. aqueous solution of methyl-green. They are straight or curved rods, which vary markedly in size and outline, and stain very irregularly. A characteristic form is a palely tinted rod with several deeply stained granules (metachromatic bodies), or with one such
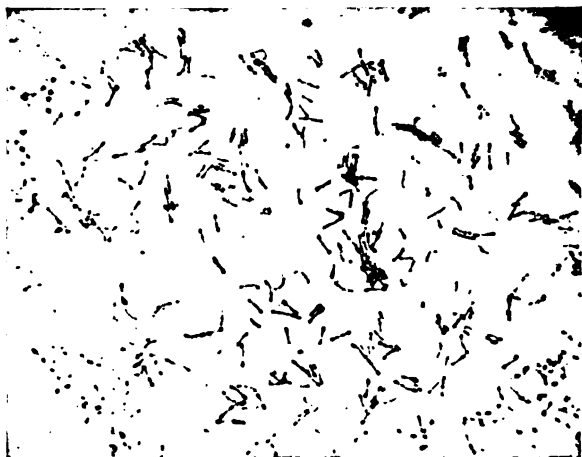
Fig. 152.—Bacillus diphtheriæ stained with methyl-green; culture from throat (× 1000) (photograph by the author).

granule at each end (Fig. 152). They stain by Gram's method. It is generally necessary, and always safer, to make a culture upon blood-serum, incubate for twelve hours, and examine smears from the growth.

Vincent's angina is a pseudomembranous and ulcerative inflammation of mouth and pharynx, which when acute may be mistaken for diphtheria, and when chronic is very apt to be mistaken for syphilis. Stained smears

from the ulcers or membrane show large numbers of spirochætæ and " fusiform bacilli," giving a striking and characteristic picture (Fig. 153). The " bacillus " is spindle shaped, more or less pointed at the ends, and about 6 to 12 μ long. The spirillum is a very slender, wavy thread, about 30 to 40 μ long. Diluted analin-gentian-violet makes a satisfactory stain. Further description is given on p. 331.
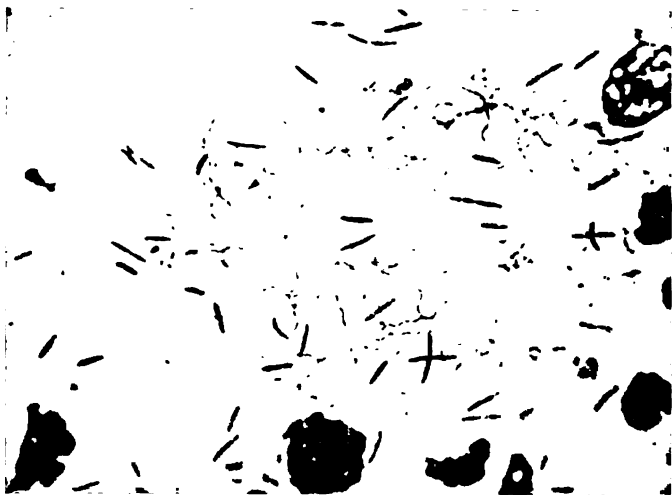
Fig. 153.—Spirochæta vincenti from case of ulcerative stomatitis ($\times$1200).

Tuberculous ulcerations of mouth and pharynx can generally be diagnosed from curetings made after careful cleansing of the surface. The curetings are well rubbed between slide and cover, and the smears thus made are dried, fixed, and stained for tubercle bacilli. Since there is much danger of contamination from tuberculous sputum, the presence of tubercle bacilli is significant only in proportion to the thoroughness with which the ulcer was

cleansed. The diagnosis is certain when the bacilli are found within groups of cells which have not been disassociated in making the smears.

THE EYE

Staphylococci, pneumococci, and *streptococci* are probably the most common of the bacteria to be found in nonspecific conjunctivitis and keratitis. Serpiginous ulcer of the cornea is generally associated with the pneumococcus (Fig. 143).

Fig. 154.—Conjunctival secretion from acute contagious conjunctivitis; polynuclear leukocytes with the bacillus of Weeks; P, phagocyte containing bacillus of Weeks (onetwelfth oil-immersion, ocular iii) (Morax).

The usual cause of acute infectious conjunctivitis ("pink-eye"), especially in cities, seems to be the *Koch-Weeks bacillus*. This is a minute, slender rod, which lies within and between the pus-corpuscles (Fig. 154), and is negative to Gram's stain. In smears it cannot be distinguished from the influenza bacillus, although its length is somewhat greater.

The *diplobacillus of Morax and Axenfeld* gives rise to an acute or chronic blepharoconjunctivitis without follicles or membrane, for which zinc sulphate seems to be a specific. It is widely distributed geographically and

is common in many regions. The organism is a short, thick diplobacillus, is frequently intracellular, and is Gram-negative (Fig. 155). A delicate capsule can sometimes be made out.

Early diagnosis of gonorrheal ophthalmia is extremely important, and can be made with certainty only by detection of *gonococci* in the discharge. They are easily found in smears from untreated cases. After treatment is

Fig. 155.—The diplobacillus of Morax and Axenfeld (from a preparation by Dr. Harold Gifford).

begun they soon disappear, even though the discharge continues.

Pseudomembranous conjunctivitis generally shows either *streptococci* or *diphtheria bacilli*. In diagnosing diphtheritic conjunctivitis, one must be on his guard against the *Bacillus xerosis*, which is a frequent inhabitant of the conjunctival sac in healthy persons, and which is identical morphologically with the diphtheria bacillus.

The clinical picture is hence more significant than the microscopic findings.

Various micro-organisms—bacteria, molds, protozoa—have been described in connection with trachoma, but the specific organism of the disease is not definitely known.

Herbert has called attention to the abundance of eosinophilic leukocytes in the discharge of *vernal catarrh*. He regards their presence in considerable numbers as very helpful in the diagnosis of this disease.

THE EAR

By far the most frequent exciting causes of acute otitis media are the pneumococcus and the streptococcus. The finding of other bacteria in the discharge generally indicates a secondary infection, except in cases complicating infectious diseases, such as typhoid fever, diphtheria, and influenza. Discharges which have continued for some time are practically always contaminated with the staphylococcus. The presence of the streptococcus should be a cause of uneasiness, since it much more frequently leads to mastoid disease and meningitis than does the pneumococcus. The staphylococcus, bacillus of Friedländer, colon bacillus, and Bacillus pyocyaneus may be met in chronic middle-ear disease.

In tuberculous disease the tubercle bacillus is present in the discharge, but its detection offers some difficulties. It is rarely easy to find, and precautions must always be taken to exclude the smegma and other acid-fast bacilli (p. 53), which are especially liable to be present in the ear. Rather striking is the tendency of old squamous cells to retain the red stain, and fragments of such cells may mislead the unwary.

PARASITIC DISEASES OF THE SKIN

Favus, tinea versicolor, and the various forms of ringworm are caused by members of the fungus group. To demonstrate them, a crust or a hair from the affected area is softened with a few drops of 20 per cent. caustic soda solution, pressed out between a slide and cover, and examined with a one-sixth objective. They consist of a more or less dense network of hyphæ and numerous round or oval refractive spores. The cuts in standard works upon diseases of the skin will aid in differentiating the members of the group.

MILK

A large number of analyses of human and cows' milk are averaged by Holt as follows, Jersey milk being excluded because of its excessive fat:

	HUMAN MILK. Normal variations, per cent.		Average, per cent.	Cows' MILK. Average, per cent.
Fat.................	3.00 to	5.00	4.00	3.50
Sugar...............	6.00 to	7.00	7.00	4.30
Proteins............	1.00 to	2.25	1.50	4.00
Salts...............	0.18 to	0.25	0.20	0.70
Water..............	89.82 to	85.50	87.30	87.50
	100.00	100.00	100.00	100.00

The reaction of human milk is slightly alkaline; of cows', neutral or slightly acid. The specific gravity of each is about 1.028 to 1.032. Human milk is sterile when secreted, but derives a few bacteria from the lacteal ducts. Cows' milk, as usually sold, contains large numbers of bacteria, the best milk rarely containing fewer than 10,000 per cubic centimeter. Microscopically,

human milk is a fairly homogeneous emulsion of fat, and is practically destitute of cellular elements.

Chemic examination of milk is of great value in solving the problems of infant feeding. The sample examined
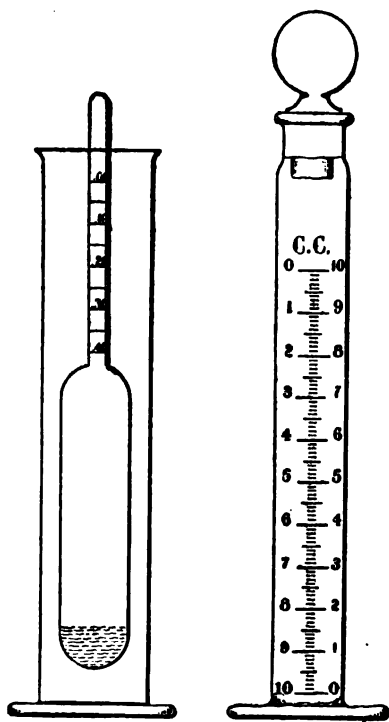
Fig. 156.—Holt's milk-testing apparatus.

should be the middle milk, or the entire quantity from one breast. The fat and protein can be estimated roughly, but accurately enough for many clinical purposes by means of Holt's apparatus, which consists of a 10 c.c. cream gage and a small hydrometer (Fig. 156). The

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cream gage is filled to the o mark with milk, allowed to stand for twenty-four hours at room temperature, and the percentage of cream then read off. The percentage of fat is three-fifths that of the cream. The protein is then approximated from a consideration of the specific gravity and the percentage of fat. The salts and sugar very seldom vary sufficiently to affect the specific gravity, hence a high specific gravity must be due to either an increase of protein or decrease of fat, or both, and vice versâ. With normal specific gravity the protein is high when the fat is high, and vice versâ. The method is not accurate with cows' milk.

For more accurate work the following methods, applicable to either human or cows' milk, are simple and satisfactory.

Fig. 157.—Tube for milk analysis.

Fat.—*Leffmann-Beam Method.*—This is essentially the widely used Babcock method, modified for the small quantities of milk obtainable from the human mammary gland. The apparatus consists of a special tube which fits the aluminum shield of the medical centrifuge (Fig. 157) and a 5 c.c. pipet. Owing to its narrow stem, the tube is difficult to fill and to clean. Exactly 5 c.c. of the milk are introduced into the tube by means of the pipet, and 1 c.c. of a mixture of equal parts of concentrated hydrochloric acid and amyl-alcohol is added and well mixed. The tube is filled to the o mark with concentrated sulphuric acid, adding a few drops at a time and agitating constantly. This is revolved in the centrifuge at 1000 revolutions a minute for three minutes, or

until the fat has separated. The percentage is then read off upon the stem, each small division representing 0.2 per cent. of fat.

Proteins.—*T. R. Boggs' Modification of the Esbach Method.*—This is applied as for urinary albumin (p. 105), substituting Boggs' reagent for Esbach's. The reagent is prepared as follows:

    (1) Phosphotungstic acid...................... 25 gm.
        Distilled water..................,............... 125 c.c.

    (2) Concentrated hydrochloric acid.............. 25 c.c.
        Distilled water............................ 100 c.c.

When the phosphotungstic acid is completely dissolved, mix the two solutions. This reagent is quite stable if kept in a dark glass bottle.

Before examination, the milk should be diluted according to the probable amount of protein, and allowance made in the subsequent reading. For human milk the optimum dilution is 1 : 10; for cows' milk, 1 : 20. Dilution must be accurate.

Lactose.—The protein should first be removed by acidifying with acetic acid, boiling, and filtering. Purdy's method may then be used as for glucose in the urine (p. 112); but it must be borne in mind that lactose reduces copper more slowly than glucose, and longer heating is, therefore, required; and that 35 c.c. of Purdy's solution is equivalent to 0.0268 gm. lactose (as compared with 0.02 gm. glucose).

It is frequently desirable to detect formalin, which is the most common preservative added to cows' milk. Add a few drops of dilute ferric chlorid solution to a few

cubic centimeters of the milk, and run the mixture gently upon the surface of some strong sulphuric acid in a test-tube.   If formaldehyd be present, a bright red ring will appear at the line of contact of the fluids.   This is not a specific test for formaldehyd, but nothing else likely to be added to the milk will give it.

SYPHILITIC MATERIAL

In 1905 Schaudinn and Hoffmann described the occurrence of a very slender, spiral micro-organism in the lesions of syphilis.   This they named *Spirochæta*

Fig. 158.—Treponema pallidum (× 1000) (Leitz ₁₂ oil-immersion objective and Leitz dark-ground condenser).

pallida, because of its low refractive power and the difficulty with which it takes up staining reagents.   The name was later changed to *Treponema pallidum.*   Its etiologic relation to syphilis is now universally admitted. It is found in primary, secondary, and tertiary lesions, but is not present in the latter in sufficient numbers to be of value in diagnosis.

Treponema pallidum is an extremely slender, spiral, motile thread, with pointed ends. There is a flagellum at each end, but it is not seen in ordinary preparations. The organism varies considerably in length, the average being about 7 μ, or the diameter of a red blood-corpuscle; and it exhibits three to twelve, sometimes more, spiral curves, which are sharp and regular and resemble the curves of a corkscrew (Figs. 112, 158, 159). It is so delicate that it is difficult to see even in well-stained
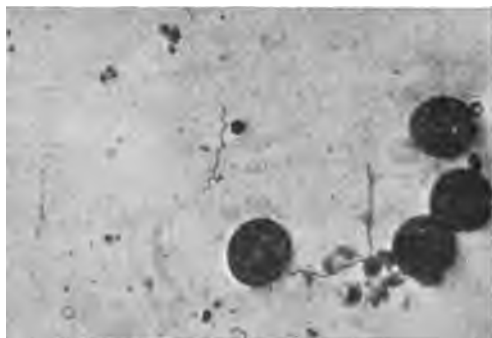
Fig. 159.—Treponema pallidum and Spirochæta refringens ($\times$ 1200) (Leitz oil-immersion objective).

preparations; a high magnification and careful focusing are, therefore, required. Upon ulcerated surfaces it is often mingled with other spiral micro-organisms, which adds to the difficulty of its detection. The most notable of these is *Spirochæta refringens*, described on p. 333.

Treponema pallidum is most easily demonstrated in chancres and mucous patches, although the skin lesions —papules, pustules, roseolous areas—often contain large numbers. Tissue-juice from the deeper portions of the lesions is the most favorable material for examination,

because the organisms are commonly more abundant than upon ulcerated surfaces and are rarely accompanied by other micro-organisms.   After cleansing, the surface is gently scraped with a curet or rubbed briskly with a swab of cotton or gauze.   In a few moments serum will exude.   The rubbing should not be so vigorous as to bring the blood, because the corpuscles may hide the treponema.   Very thin cover-glass smears are then made from the serum.

Staining Methods.—Giemsa's stain is probably the most widely used.   It is best purchased ready prepared.   Smears   . are fixed in absolute alcohol for fifteen minutes.   Ten drops of the stain are added to 10 c.c. of faintly alkaline distilled water (1 drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of the water), and the fixed smear is immersed in this diluted stain for one to three hours or longer.   It is then rinsed in distilled water, dried, and mounted.   In well-stained specimens *Treponema pallidum* is reddish, most other micro-organisms, bluish.   More intense staining may be obtained by gently warming the stain.

Wright's blood-stain, used in the manner already described (p. 222) except that the diluted stain is allowed to act upon the film for fifteen minutes, gives good results.

Silver Method.—The silver impregnation method has long been used for tissues.   Stein has applied it to smears as follows:

1. Dry the films in the incubator at 37° C. for three or four hours.

2. Immerse in 10 per cent. silver nitrate solution, in diffuse daylight for some hours, until the preparation takes on a metallic luster.

3. Wash in water, dry, and mount.

The organisms are black against a brownish background.

India-ink Method.—A drop of India-ink of good grade (Günther and Wagner's recommended) is diluted with 1 to 5 drops of water. A loopful of this is mixed on a slide with a similar quantity of serum from the suspected lesion. The mixture is then spread over the slide and allowed to dry. After drying, it is examined with an oil-immersion lens. Micro-organisms, including *Treponema pallidum*, appear clear white on a brown or black background, much as they do with the dark ground condenser (Fig. 158). Because of its extreme simplicity this method has been favorably received. It cannot, however, be absolutely relied upon, since, as has been pointed out, many India-inks contain wavy vegetable fibrils which might easily mislead a beginner, and sometimes, indeed, even an experienced worker.

Dark ground illumination (see p. 21) may be used to study the living organisms in fresh tissue juices. This offers a satisfactory means of diagnosis, but since the instrument is expensive the practitioner will rely upon one or more of the staining methods just enumerated.

SEMEN

Absence of spermatozoa is a more common cause of sterility than is generally recognized. In some cases they are present, but lose their motility immediately after ejaculation.

Semen must be kept warm until examined. When it must be transported any considerable distance, the method suggested by Boston is convenient. The fresh semen is placed in a small bottle, to the neck of which a string is attached. This is then suspended from a button on the trousers, so that the bottle rests against the skin of the inguinal region. It may be carried in this way for hours. When ready to examine, place a small quantity

upon a warmed slide and apply a cover. The spermatozoa are readily seen with a 4 mm. objective (Fig. 57). Normally, they are abundant and in active motion.

Detection of semen in stains upon clothing, etc., is often important. The finding of spermatozoa, after soaking the stain for an hour in normal salt solution or
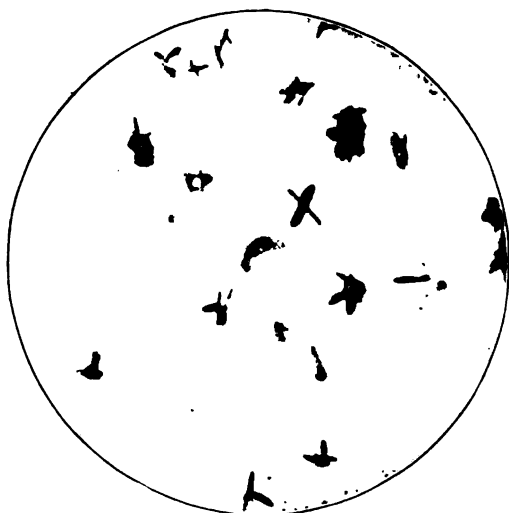
Fig. 160.—Seminal crystals (medium size) (×750) from a stain on clothing. A single thread ½ inch long was used in the test, the stain being three years and four months old (Peterson and Haines).

dilute alcohol, and teasing in the same fluid, is absolute proof that the stain in question is semen, although it is not possible to distinguish human semen from that of the lower animals in this way. A little eosin added to the fluid will bring the spermatozoa out more clearly.

Florence's Reaction.—The suspected material is softened with water, placed upon a slide with a few drops

of the reagent, and examined at once with a medium power of the microscope. If the material be semen, there will be found dark-brown crystals (Fig. 160) in the form of rhombic platelets resembling hemin crystals, or of needles, often grouped in clusters. These crystals can also be obtained from crushed insects, watery extracts of various internal organs, and certain other substances, so that they are not absolute proof of the presence of semen. Negative results, upon the other hand, are conclusive, even when the semen is many years old.

The reagent consists of iodin, 2.54 gm.; potassium iodid, 1.65 gm.; and distilled water, 30 c.c.

DIAGNOSIS OF RABIES

In view of the brilliant results attending prophylactic treatment by the Pasteur method, early diagnosis of rabies (hydrophobia) in animals which have bitten persons is extremely important.

The most reliable means of diagnosis is the production of the disease in a rabbit by subdural or intracerebral injection of a little of the filtrate from an emulsion of the brain and medulla of the suspected animal. The diagnosis can, however, usually be quickly and easily made by microscopic demonstration of Negri bodies. Whether these bodies be protozoan in nature and the cause of the disease, as is held by many, or whether they be products of the disease, it is certain that their presence is pathognomonic.

Negri bodies are sharply outlined, round, oval, or somewhat irregular structures which vary in size, the extremes being 0.5 and 18 μ. They consist of a hyalin-like cytoplasm, in which when properly stained one or

more chromatin bodies can usually be seen. They are situated chiefly within the cytoplasm of the large cells of the central nervous system, the favorite locations being the multipolar cells of the hippocampus major (Ammon's horn). In many cases they suggest red blood-corpuscles lying within nerve-cells.

Probably the best method of demonstrating Negri bodies is the impression method of Langdon Frothingham, which is carried out as follows:

(1) Place the dog's brain[1] upon a board about 10 inches square, and divide into two halves by cutting along the me-· dian line with scissors.

(2) From one of the halves cut away the cerebellum and open the lateral ventricle, exposing the Ammon's horn.

(3) Dissect out the Ammon's horn as cleanly as possible.

(4) Cut out a small disc at right angles to the long axis of the Ammon's horn, so that it represents a cross-section of the organ.

(5) Place this disc upon the board near the edge, with one of the cut surfaces upward.

(6) Press the surface of a thoroughly clean slide upon the disc and lift it suddenly. The disc (if its exposed surface has not been allowed to become too dry) will cling to the board, leaving only an impression upon the slide. Make several similar impressions upon different portions of the slide, using somewhat greater pressure each time. Impressions are also to be made from the cut surface of the cerebellum, since Negri bodies are sometimes present in the Purkinje cells when not found in the Ammon's horn.

(7) Before the impressions dry, immerse in methyl-alcohol for one-half to two minutes.

[1] For Dr. Frothingham's method of removing a dog's brain see American Journal of Public Hygiene for February, 1908.

PLATE XIII
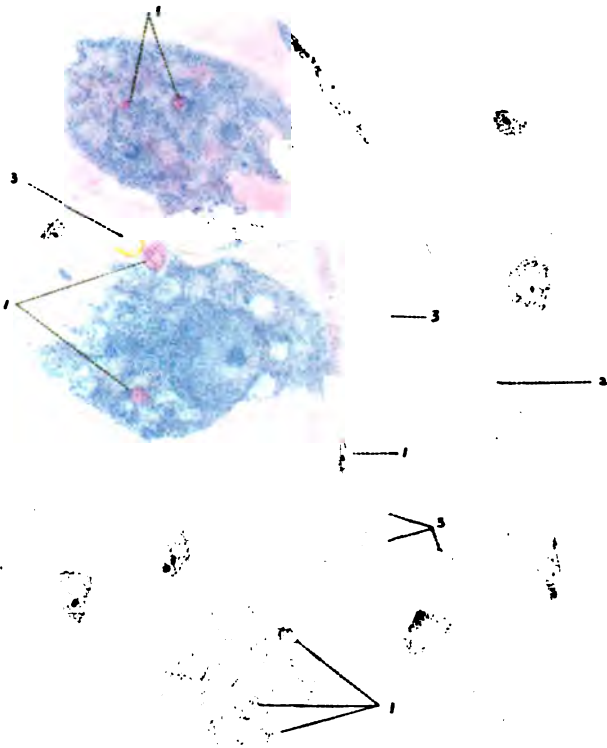
Nerve-cells containing Negri bodies.

Hippocampus impression preparation, dog. Van Gieson stain; × 1000. 1, Negri bodies; 2, capillary; 3, free red blood-corpuscles (courtesy of Langdon Frothingham).

(8) Cover with Van Gieson's methylene-blue-fuchsin stain, warming gently for one-half to two minutes. This stain, as modified by Frothingham, is as follows. It must be freshly mixed each day:

Tap-water.................................20 c.c.
Saturated alcoholic solution basic fuchsin ...... 3 drops.
Saturated aqueous solution methylene-blue..... 1 drop.

(9) Wash in water and dry with filter-paper. Examine with a low power to locate the large cells in which the bodies are apt to be found, and study these with an oil-immersion lens.

The Negri bodies are stained a pale pink to purplish red, and frequently contain small blue dots (Plate XIII). The nerve-cells are blue, and red blood-corpuscles are colorless or yellowish-copper colored.

When the work is finished, the board with the dissected brain is sterilized in the steam sterilizer.

Demonstration of Negri bodies by this method is quicker and, probably, more certain than by the study of sections. It has the decided advantage over the smear method that the histologic structure is retained. One or more of the impressions generally shows the entire cell arrangement almost as well as in sections, and it is very easy to locate favorable fields with a 16 mm. objective.

CHAPTER VIII

BACTERIOLOGIC METHODS

BACTERIOLOGY has become so important a part of medicine that some knowledge of bacteriologic methods is imperative for the present-day practitioner. It has been the plan of this book to describe the various bacteria and bacteriologic methods with the subjects to which they seemed to be particularly related. The tubercle bacillus and its detection, for example, are described in the chapters upon Sputum and Urine; blood-cultures are discussed in the chapter upon Blood. There are, however, certain methods, notably the preparation of media and the study of bacteria by cultures, which do not come within the scope of any previous section, and an outline of these is given in the present chapter.

I. APPARATUS

Much of the apparatus of the clinical laboratory is called into use. Only the following need special mention:

1. **Sterilizers.**—Two are required.

The *dry*, or *hot-air sterilizer*, is a double-walled oven similar to the detached ovens used with gas and gasolene stoves. It has a hole in the top for a perforated cork with thermometer.

The *steam sterilizer* is preferably of the Arnold type, opening either at the top or the side. An *autoclave*, which

sterilizes with steam under pressure, is very desirable, but not necessary.

2. **Incubator.**—This is the most expensive piece of apparatus which will be needed. It is made of copper, and has usually both a water- and an air-jacket surrounding the incubating chamber. It is provided with thermometer, thermo-regulator, and some source of heat, usually a Koch safety Bunsen burner. With a little ingenuity one can rig up a drawer or a small box, in which a fairly constant temperature can be maintained by means of an electric light. The degree of heat can be regulated by moving the drawer in or out, or holes can be made in which corks may be inserted and removed as needed. A Thermos bottle has been suggested as a temporary make-shift.

3. **Culture-tubes and Flasks.**—For most work ordinary test-tubes, 5 by ¾ inches, are satisfactory. For special purposes a few 3 by ½ inch and 6 by ¾ inch tubes may be needed. Heavy tubes, which do not easily break, can be obtained, and are especially desirable when tubes are cleaned by an untrained assistant.

Flasks of various sizes are needed. The Ehrlenmeyer type is best. Quart and pint milk bottles and 2-ounce wide-mouthed bottles will answer for most purposes.

4. **Platinum Wires.**—At least two of these are needed. Each consists of a piece of platinum wire about 8 cm. long, fixed in the end of a glass or metal rod. One is made of about 22 gage wire and its end is curled into a loop 1 to 2 mm. in diameter. A loop 1 mm. in diameter is sometimes called a "normal." The other wire is somewhat heavier and its tip is hammered flat.

5. **Pipets, etc.**—In addition to the graduated pipets with which every laboratory is supplied, there are a number of forms which are generally made from glass tubing as needed. One of the simplest of these is made as follows: A section of glass tubing, about 12 cm. long and 8 mm. in diameter, is grasped at the ends, and its center is heated in a concentrated flame. A blast-lamp is best, but a Bunsen burner will usually answer, par-
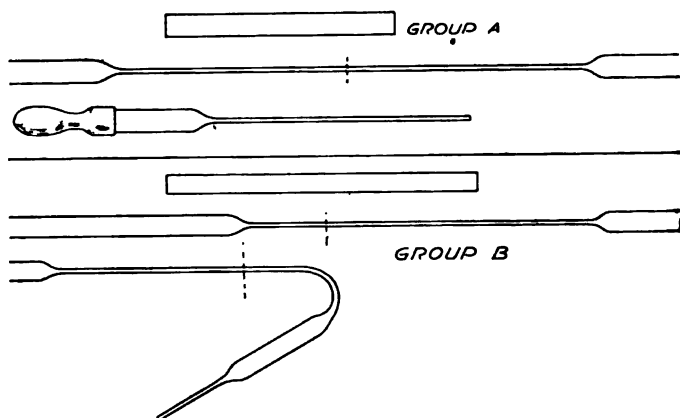
Fig. 161.—Process of making pipets (group A) and Wright's capsule (group B). The dotted lines indicate where the glass is to be broken.

ticularly if fitted with a "wing" attachment. When the glass is thoroughly softened it is removed from the flame, and, with a steady, but not rapid pull, is drawn out as shown in Fig. 161. The slender portion is scratched near the middle with a file and is broken to make two pipets, which are then fitted with rubber nipples. Two conditions are essential to success: the glass must be thoroughly softened and it must be removed from the flame before beginning to pull.

A nipple can be made of a short piece of rubber tubing, one end of which is plugged with a glass bead.

This pipet has many uses about the laboratory. When first made it is sterile and may be used to transfer cultures. With a grease-pencil mark about 2 cm. from its tip (Fig. 163), it is useful for measuring very small quantities of fluid, as in making dilutions for the Widal test and in counting bacteria in vaccines. Mett's tubes for pepsin estimation may be made from the capillary portion. The capillary portion also makes a very satisfactory blood-lancet if heated in a low flame and drawn out quickly.

Another useful device is the Wright capsule, which is made as shown in Fig. 161. Its use is illustrated in Fig. 100. After the straight end is sealed the curved portion may be hooked over the aluminum tube of the centrifuge, and the contained blood or other fluid sedimented; but the speed should not be so great as to break the capsule.

II. STERILIZATION

All apparatus and materials used in bacteriologic work must be sterilized before use.

Glassware, metal, etc., are heated in the hot-air sterilizer at 150° to 180° C. for half an hour. Flasks, bottles, and tubes are plugged with cotton before heating. Petri dishes may be wrapped in paper in sets of three. Pipets and glass and metal hypodermic syringes are placed in cotton-stoppered test-tubes.

Culture-media and other fluids must be sterilized by steam. Exposure in an autoclave to a temperature of 110° C. (6 pounds pressure) for one-half hour is sufficient. With the Arnold sterilizer, which is more commonly

used, the intermittent plan must be adopted, since steam at ordinary pressure will not kill spores. This consists in steaming for thirty to forty-five minutes on three or four successive days. Spores which are not destroyed upon the first day develop into the vegetative form and are destroyed at the next heating. Gelatin media must not be exposed to steam for more than twenty minutes at a time, and must then be removed from the sterilizer and cooled in cold water, otherwise the gelatin may lose its power to solidify.

Cotton and **gauze** are sterilized by either hot air or steam, preferably the latter.

III. PREPARATION OF CULTURE-TUBES

New tubes should be washed in a very dilute solution of nitric acid, rinsed in clear water, and allowed to drain dry.

Tubes which contain dried culture-media are cleaned with a test-tube brush after boiling in a strong solution of washing-soda. They are then rinsed successively in clear water, acidulated water, and clear water, and allowed to drain.

The tubes are now ready to be plugged with raw cotton—the "cotton batting" of the dry goods stores. This is done by pushing a wad of cotton into each tube to a depth of about 3 cm. with a glass rod. The plugs should fit snugly, but not too tightly, and should project from the tube sufficiently to be readily grasped by the fingers. The tubes are next placed in wire baskets and heated in an oven for about one-half hour at 150° C. in order to mold the stoppers to the shape of the tubes. The heating should not char the cotton, although a slight brown-

ing does no harm. The tubes are now ready to be filled with culture-media.

IV. CULTURE-MEDIA

For a careful study of bacteria a great variety of culture-media is required, but only a few—bouillon, agar or solidified blood-serum, and gelatin—are much used in routine work.

Preparation of Culture-media.—

BEEF INFUSION

Hamburger steak, lean..................... 500 grams.
Tap-water................................ 1000 c.c.

Mix well; let soak about twenty-four hours in an ice-chest, and squeeze through cheese-cloth. This infusion is not used by itself, but forms the basis for various media. "Double strength" infusion, used in making agar-agar, requires equal parts of the meat and water.

INFUSION BOUILLON

Beef infusion............................ 1000 c.c.
Peptone (Witte).......................... 10 grams.
Salt..................................... 5 "

Boil until dissolved; bring to original bulk with water; adjust reaction; and filter.

BEEF EXTRACT BOUILLON

Liebig's extract of beef.................... 3 grams.
Peptone................................. 10 "
Salt..................................... 5 "
Tap-water............................... 1000 c.c.

When all ingredients are dissolved, cool, and beat in the whites of two eggs; boil briskly for five minutes and filter. It is not usually necessary to adjust the reaction.

26

AGAR-AGAR

Preparation of this medium usually gives the student much trouble. There should be no difficulty if the directions are carefully carried out.

Agar-agar, powdered or in shreds............	15 grams.
Tap-water...............................	500 c.c.

Boil until thoroughly dissolved and add—

Peptone...............................	10 grams
Salt..................................	5 "

When these have dissolved, replace the water lost in boiling, cool to about 60° C., and add 500 c.c. double-strength beef infusion. Bring slowly to the boil, adjusting the reaction meanwhile, and boil for at least five minutes. Filter while *hot* through a moderately thick layer of absorbent cotton wet with *hot* water in a *hot* funnel. A piece of coarse wire gauze should be placed in the funnel underneath the cotton to give a larger filtering surface. This medium will be clear enough for ordinary work. If an especially clear agar is desired, it can be filtered through paper in an Arnold sterilizer.

Agar can also be made by boiling 15 grams of powdered agar in 1000 c.c. of bouillon until dissolved, replacing the water lost in boiling, and filtering through paper in a sterilizer. It can be cleared with egg if desired.

GLYCERIN AGAR-AGAR

To 1000 c.c. melted agar add 60 to 70 c.c. glycerin.

GELATIN

Dissolve 100 to 120 grams "golden seal" gelatin in 1000 c.c. nutrient bouillon with as little heat as possible,

adjust the reaction, cool, beat in the whites of two eggs, boil, and filter hot through filter-paper wet with hot water. Sterilize in an Arnold sterilizer for twenty minutes upon three successive days and cool in cold water after each heating.

Sugar Media

Any desired sugar may be added to bouillon, agar, or gelatin in proportion of 10 grams to the liter. Dextrose is most frequently required. When other sugars are added, media made from beef-extract should be used, since those made from beef-infusion contain enough dextrose to cause confusion.

Löffler's Blood-serum

1 per cent. dextrose-bouillon.............	1 part.
Blood-serum.............................	3 parts.

Mix and tube. Place in an inspissator at the proper slant for three to six hours at 80° to 90° C. When firmly coagulated, sterilize in the usual way. A large "double-cooker" makes a satisfactory inspissator. The tubes are placed in the inner compartment at the proper slant, a lid with perforation for a thermometer is applied, and the whole is weighted down in the water of the outer compartment.

Blood-serum is obtained as follows: Beef or pig blood is collected in a bucket at the slaughter-house and placed in an ice-chest until coagulated. The clot is then gently loosened from the wall of the vessel. After about twenty-four hours the serum will have separated nicely and can be siphoned off. It is then stored in bottles with a little chloroform until needed. Red cells, if abundant, darken the medium, but do no harm.

Solidified blood-serum is probably the most satisfactory medium for general purposes. Nearly all pathogenic organisms grow well upon it.

HEMOGLOBIN MEDIUM

The simplest way to prepare this is to smear a drop of blood, obtained by puncture of the finger, over the surface of an agar-slant, and to incubate over night to make sure of sterility. It is used for growing the influenza bacillus.

LITMUS MILK

Fresh milk is steamed in an Arnold sterilizer for half an hour, and placed in the ice-chest over night. The milk is siphoned off from beneath the cream, and sufficient aqueous solution of litmus or, preferably, azolitmin is added to give a blue-violet color. It is then tubed and sterilized.

POTATO

Cylinders about one-half inch thick are cut from potato and split obliquely. These are soaked over night in running water and placed in large tubes, in the bottom of which is placed a little cotton saturated with water. They are sterilized for somewhat longer periods than ordinary media.

DUNHAM'S PEPTONE SOLUTION

Peptone...............................	10 grams.
Salt..................................	5 "
Water................................	1000 c.c.

Dissolve by boiling; filter, tube, and sterilize.

This medium is used to determine indol production. To a twenty-four- to forty-eight-hour-old culture is

added 5 to 10 drops of concentrated c. p. sulphuric acid and 1 c.c. of 1 : 10,000 solution of sodium nitrite. Appearance of a pink color shows the presence of indol. A pink color before the nitrite is added shows the presence of both indol and nitrites.

HISS' SERUM MEDIA

Blood-serum.............................. 1 part.
Water.................................... 3 parts.

Warm and adjust reaction to + 0.2 to + 0.8. Add litmus or azolitmin solution to give a blue-violet color. Finally, add 1 per cent. of inulin or any desired sugar. The inulin medium is very useful in distinguishing between the pneumococcus and streptococcus.

BILE MEDIUM

Ox- or pig-bile is obtained at the slaughter-house, tubed, and sterilized. This is used especially for growing typhoid bacilli from the blood during life. The following is probably as satisfactory as fresh bile and is more convenient:

Inspissated ox-bile (Merck)............ 30.0 grams.
Peptone................................ 2.5   "
Water .... 250.0 c.c.

Dissolve, place in tubes, and sterilize.

Reaction of Media.—The chemic reaction of the medium exerts a marked influence upon the growth of bacteria. It is adjusted after all ingredients are dissolved by adding sufficient caustic soda solution to overcome the acidity of the meat and other substances used. In general, the most favorable reaction lies between the neutral points of litmus and phenolphtha-

lein, representing a very faint alkalinity to litmus. In routine work it is usually sufficient to test with litmus-paper. When greater accuracy is demanded, the following method should be used: After all ingredients are dissolved and the loss during boiling has been replaced with water, 10 c.c. of the medium are transferred to an evaporating dish, diluted with 40 c.c. of water, and boiled for three minutes to drive off carbon dioxid. One c.c. of 0.5 per cent. alcoholic solution of phenolphthalein is then added, and decinormal sodium hydroxid solution is run in from a buret until the neutral point is reached, indicated by the appearance of a permanent pink color. The number of cubic centimeters of decinormal solution required to bring this color indicates the number of cubic centimeters of *normal* sodium hydroxid solution which will be required to neutralize 100 c.c. of the medium. The standard reaction is +1.5, which means that the medium must be of such degree of acidity that 1.5 c.c. of normal solution would be required to neutralize 100 c.c. This corresponds to faint alkalinity to litmus. Most pathogenic bacteria grow better with a reaction of +1.0 or +0.8. Example: If the 10 c.c. which were titrated required 2 c.c. of decinormal solution to bring the pink color, the reaction is +2; and 0.5 c.c. of normal sodium hydroxid must be added to each 100 c.c. of the medium to reduce it to the standard, +1.5.

Tubing Culture-media.—The finished product is stored in flasks or distributed into test-tubes. This is done by means of a funnel fitted with a section of rubber tubing with a glass tip and a pinch-cock. Great care must be exercised, particularly with media which solidify, not to smear any of it upon the inside of the mouth of

the tube, otherwise the cotton stopper will stick. Tubes are generally filled to a depth of 3 or 4 cm. For stab-cultures a greater depth is desired.

After tubing, all culture-media must be sterilized as already described. Agar-tubes are cooled in a slanting position to secure the proper surface for inoculation.

Media should be stored in a cool place, preferably an ice-chest. Evaporation may be prevented by covering the tops of the tubes with tin-foil or the rubber caps which are sold for the purpose; or the cotton stopper may be pushed in a short distance and a cork inserted.

V. STAINING METHODS

In general, bacteria are stained to determine their morphology, their reaction with special methods (e. g., Gram's method), and the presence or absence of certain structures, as spores, flagella, and capsules. Staining methods for various purposes and the formulæ of the staining fluids have been given in previous chapters and can be found by consulting the index. The following will probably be most frequently used:

Methods for tubercle bacilli, pp. 49, 51, and 168.
Methods for capsules of bacteria, pp. 55 and 368.
Methods for *Treponema pallidum*, p. 390.
Löffler's alkaline methylene-blue, p. 57.
Blood-stains, pp. 221–224.

The method of staining bacteria for morphology is as follows, using any simple bacterial stain:

(1) Make a thin smear upon a slide or cover-glass.
(2) Dry in the air, or by warming high above the flame.

(3) " Fix " by passing the preparation, film side up, rather slowly through the flame of a Bunsen burner: a cover-glass three times, a slide about twelve times. Take care not to scorch.

(4) Apply the stain for the necessary length of time, generally one-quarter to one minute.

(5) Wash in water.

(6) Dry by waving high above a flame or by blotting with filter-paper.

(7) Mount by pressing the cover, film side down, upon a drop of Canada balsam on a slide. Slides may be examined with the oil-immersion lens without a cover-glass.

Simple Bacterial Stains.—A simple solution of any basic anilin dye (methylene-blue, basic fuchsin, gentian violet, etc.) will stain nearly all bacteria. These simple solutions are not much used in the clinical laboratory, because other stains, such as Löffler's methylene-blue and Pappenheim's pyronin-methyl-green stain, which serve the purpose even better, are at hand.

Pappenheim's Pyronin-methyl-green Stain.—This solution colors bacteria red and nuclei of cells blue. It is, therefore, especially useful for intracellular bacteria like the gonococcus and the influenza bacillus. It is a good stain for routine purposes, and is a most excellent contrast stain for Gram's method. It colors the cytoplasm of lymphocytes bright red, and has been used as a differential stain for these cells. The solution is applied cold for one-half to five minutes. It consists of saturated aqueous solution methyl-green, 3 to 4 parts, and saturated aqueous solution pyronin, 1 to 1½ parts.

Carbol Thionin.—Saturated solution thionin in 50 per

cent. alcohol, 20 c.c.; 2 per cent. aqueous solution phenol, 100 c.c.

This is especially useful in counting bacteria for a vaccine (p. 422). It can be used as a general stain. In blood work it is used for the malarial parasite and for demonstration of basophilic degeneration of the red cells. It should be preceded by fixation for about a minute in saturated aqueous solution of mercuric chlorid.

Gram's Method.—This is a very useful aid in differentiating certain bacteria and should be frequently resorted to. It depends upon the fact that when treated successively with gentian-violet and iodin, certain bacteria (owing to formation of insoluble compounds) retain the stain when treated with alcohol, whereas others quickly lose it. The former are called *Gram-positive*, the latter, *Gram-negative*. In order to render Gram-negative organisms visible, some contrasting counterstain is commonly applied, but this is not a part of Gram's method proper.

(1) Make smears, dry, and fix by heat.

(2) Apply anilin-gentian-violet or formalin-gentian-violet (p. 57) two to five minutes.

(3) Wash with water.

(4) Apply Gram's iodin solution one-half to two minutes.

(5) Wash in alcohol until the purple color ceases to come off. This is conveniently done in a watch-glass. The preparation is placed in the alcohol, face downward, and one edge is raised and lowered with a needle. As long as any color is coming off, purple streaks will be seen diffusing into the alcohol where the surface of the fluid meets the smear. If forceps be used, beware of stain which may have dried

upon them. The thinner portions of smears from pus should be practically colorless at this stage.

(6) Apply a contrast stain for one-half to one minute. The stains commonly used for this purpose are an aqueous or alcoholic solution of Bismarck brown and a weak solution of fuchsin. In the writer's experience, Pappenheim's pyronin-methyl-green mixture is much more satisfactory; it brings out Gram-negative bacteria more sharply, and is especially desirable for intracellular Gram-negative organisms like the gonococcus and influenza bacillus, since the bacteria are bright red and nuclei of cells blue.

(7) Wash in water, dry, and mount in balsam.

The more important bacteria react to this staining method as follows:

GRAM STAINING (Deep purple).	GRAM DECOLORIZING (Colorless unless a counterstain is used).
Staphylococcus.	Gonococcus.
Streptococcus.	Meningococcus.
Pneumococcus.	Micrococcus catarrhalis.
Bacillus diphtheriæ.	Bacillus of influenza.
Bacillus tuberculosis.	Typhoid bacillus.
Bacillus of anthrax.	Bacillus coli communis.
Bacillus of tetanus.	Spirillum of Asiatic cholera.
Bacillus aërogenes capsulatus.	Bacillus pyocyaneus.
	Bacillus of Friedländer.
	Koch-Weeks bacillus.
	Bacillus of Morax-Axenfeld.

Moeller's Method for Spores.—Bodies of bacteria are blue, spores are red.

(1) Make thin smears, dry, and fix.

(2) Wash in chloroform for two minutes.

(3) Wash in water.

(4) Apply 5 per cent. solution of chromic acid one-half to two minutes.

(5) Wash in water.

(6) Apply carbol-fuchsin and heat to boiling.

(7) Decolorize in 5 per cent. solution of sulphuric acid.

(8) Wash in water.

(9) Apply 1 per cent. aqueous solution of methylene-blue one-half minute.

(10) Wash in water, dry, and mount.

Löffler's Method for Flagella.—The methods for flagella are applicable only to cultures. Enough of the growth from an agar-culture (which should not be more than eighteen to twenty-four hours old) to produce faint cloudiness is added to distilled water. A small drop of this is placed on a cover-glass, spread by tilting, and dried quickly. The covers must be absolutely free from grease. To insure this, they may be warmed in concentrated sulphuric acid, washed in water, and kept in a mixture of alcohol and strong ammonia. When used they are dried upon a fat-free cloth.

(1) Fix by heating the cover over a flame while holding in the fingers.

(2) Cover with freshly filtered mordant and gently warm for about a minute.

The mordant consists of:

20 per cent. aqueous solution of tannic acid.......... 10 c.c.
Saturated solution ferrous sulphate, cold............. 5 c.c.
Saturated aqueous or alcoholic solution gentian violet.. 1 c.c.

(3) Wash in water.

(4) Apply freshly filtered anilin-gentian-violet, warming gently for one-half to one minute.

(5) Wash in water, dry, and mount in balsam.

VI. METHODS OF STUDYING BACTERIA

The purpose of bacteriologic examinations is to determine whether bacteria are present or not, and, if present, their species and comparative numbers. In general, this is accomplished by: 1, Direct microscopic examination; 2, Cultural methods; 3, Animal inoculation.

1. Microscopic Examination.—Every bacteriologic examination should begin with a microscopic study of smears from the pathologic material, stained with a general stain, by Gram's method, and often by the method for the tubercle bacillus. This yields a great deal of information to the experienced worker, and in many cases is all that is necessary for the purpose in view. It will · at least give a general idea of what is to be expected, and may determine future procedure.

2. Cultural Methods.—(1) **Collection of Material.**— Material for examination must be collected under absolutely aseptic conditions. It may be obtained with a platinum wire—which has been heated to redness just previously and allowed to cool—or with a swab of sterile cotton on a stiff wire or wooden applicator. Such swabs may be placed in cotton-stoppered test-bubes, sterilized, and kept on hand ready for use. Fluids which contain very few bacteria, and hence require that a considerable quantity be used, may be collected in a sterile hypodermic syringe or one of the pipets described on p. 398. The method of obtaining blood for cultures is given on p. 245.

(2) **Inoculating Media.**—The material is thoroughly distributed over the surface of some solid medium, solid-

ified blood-serum being probably the best for routine work. When previous examination of smears has shown that many bacteria are to be expected, a second tube should be inoculated from the first, and a third from the second, so as to obtain isolated colonies in at least one of the tubes. The platinum wire must be heated to redness before and after each inoculation. When only a few organisms of a single species are expected, as is the case in blood-cultures, a considerable quantity of the material is mixed with a fluid medium.

(3) **Incubation.**—Cultures are placed in an incubator which maintains a uniform temperature, usually of 37.5° C., for eighteen to twenty-four hours, and the growth, if any, is studied as described later. Gelatin will melt with this degree of heat, and must be incubated at about room-temperature.

(4) **Study of Cultures.**—When the original . culture contains more than one species, they must be separated, or obtained in "pure culture," before they can be studied satisfactorily. To accomplish this it is necessary to so distribute them on solid media that they form separate colonies, and to inoculate fresh tubes from the individual colonies. In routine work the organisms can be sufficiently distributed by drawing the infected wire over the surface of the medium in a series of streaks. If a sufficient number of streaks be made, some of them are sure to show isolated colonies. Another method of obtaining isolated colonies is to inoculate the water of condensation of a series of tubes, the first from the second, the second from the third, etc., and, by tilting, to flow the water once over the surface of the medium.

In order to determine the species to which an organism belongs it is necessary to consider some or all of the following points:

(1) Naked-eye and microscopic appearance of the colonies on various media.

(2) Comparative luxuriance of growth upon various media. The influenza bacillus, for example, can be grown upon media containing hemoglobin, but not on the ordinary media.

(3) Morphology, special staining reactions, and the presence or absence of spores, flagella, and capsules. Staining methods for these purposes have been given.

(4) Motility. This is determined by observing the living organism with an oil-immersion lens in a hanging-drop preparation, made as follows: A small drop of a bouillon culture or of water of condensation from an agar or blood-serum tube is placed upon the center of a cover-glass; this is inverted over the concavity of a "hollow slide," and ringed with vaselin. In focusing, the edge of the drop should be brought into the field. Great care must be exercised not to break the cover by pushing the objective against it. A method which in some respects is preferable to the hanging drop is to make a ring of vaselin on a slide, place a drop of the culture in this, and apply a cover.

It is not always easy to determine whether an organism is or is not motile, since the motion of currents and the Brownian motion which affects all particles in suspension are sometimes very deceptive.

(5) Production of chemic changes in the media. Among these are coagulation of milk; production of acid in milk and various sugar media to which litmus has been

added to detect the change; production of gas in sugar media, the bacteria being grown in fermentation tubes similar to those used for sugar tests in urine; and production of indol.

(6) Ability to grow without oxygen. For anaërobic methods, the reader is referred to larger works.

(7) Effects produced when inoculated into animals.

3. Animal Inoculation.—In clinical work this is resorted to chiefly to detect the tubercle bacillus. The method is described on p. 375.

For the study of bacteria in cultures, a small amount of a pure culture is injected subcutaneously or into the peritoneal cavity. The animals most used are guinea-pigs, rabbits, and mice. For intravenous injection the rabbit is used because of the easily accessible marginal vein of the ear.

VII. CHARACTERISTICS OF SPECIAL BACTERIA

Owing to the great number of bacterial species, most of which have not been adequately studied, positive identification of an unknown organism is often a very difficult problem. Fortunately, however, only a few are commonly encountered in routine work, and identification of these with comparative certainty presents no great difficulty. Their more distinctive characteristics are outlined in this section.

1. **Staphylococcus Pyogenes Aureus.**—The morphology and staining reactions (described on p. 368) and the appearance of the colonies are sufficient for diagnosis. Colonies on solidified blood-serum and agar are rounded, slightly elevated, smooth and shining, and vary in color

from light yellow to deep orange. Young colonies are sometimes white.

2. **Staphylococcus Pyogenes Albus.**—This is similar to the above, but colonies are white. It is generally less virulent.

3. **Staphylococcus Pyogenes Citreus.**—The colonies are lemon yellow; otherwise it resembles the white staphylococcus.

4. **Streptococcus Pyogenes.**—The morphology and staining reactions have been described (p. 368). The chains are best seen in the water of condensation and in bouillon cultures. The cocci are not motile. Colonies on blood-serum are minute, round, grayish, and translucent. Litmus milk is usually acidified and coagulated, although slowly. The streptococcus rarely produces acid in Hiss' serum-water-litmus-inulin medium (see p. 405).

5. **Pneumococcus.**—The only organism with which this is likely to be confused is the streptococcus. The distinction is often extremely difficult.

Detection of the pneumococcus in fresh material has been described (p. 54). In cultures it frequently forms long chains. Capsules are not present in cultures except upon special media. They show best upon a serum medium like that described for the gonococcus, but can frequently be seen in milk. Colonies on blood-serum resemble those of the streptococcus. The pneumococcus usually promptly acidifies and coagulates milk, and acidifies and coagulates Hiss' serum-water with inulin. The latter property is very helpful in diagnosis.

6. **Gonococcus.**—Its morphology and staining pecu-

liarities are given on p. 369. These usually suffice for its identification, cultural methods being rarely undertaken. In cultures the chief diagnostic point is its failure to grow on ordinary media. To grow it the most convenient medium is made by adding ascitic or hydrocele fluid (which has been obtained under aseptic conditions) to melted agar in proportion of 1 part of serum to 3 parts of agar. The agar in tubes is melted and cooled to about 45° C.; the serum is added with a pipet and mixed by shaking; and the tubes are again cooled in a slanting position. Colonies upon this medium are minute, grayish, and translucent.

7. **Diplococcus Intracellularis Meningitidis.**—It grows poorly or not at all on plain agar. On Löffler's blood-serum, upon which it grows fairly well, colonies are round, colorless or hazy, flat, shining, and viscid looking. It quickly dies out.

8. **Diphtheria Bacillus.**—The diagnosis is usually made from a study of stained smears from cultures upon blood-serum. Its morphology and staining peculiarities are characteristic when grown on this medium (see p. 379). The bacilli are non-motile and Gram-positive. The colonies are round, elevated, smooth, and grayish.

9. **Typhoid and Colon Bacilli.**—These are medium-sized, motile, Gram-negative, non-spore-bearing bacilli. Upon blood-serum they form rounded, grayish, slightly elevated, viscid looking colonies, those of the colon bacillus being somewhat the larger. They do not liquefy gelatin. They represent the extremes of a large group with many intermediate types. They are distinguished as follows:

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Typhoid Bacillus.	*Colon Bacillus.*
Actively motile.	Much less active.
Growth on potato usually invisible.	Growth distinctly visible as dirty gray or brownish slimy layer.
No gas produced in glucose media.	Produces gas.
Growth in litmus milk produces no change.	Litmus milk pink and coagulated.
Produces no indol in Dunham's peptone medium.	Produces indol. (For test, see p. 404.)
Agglutinates with serum from typhoid-fever patient. (Recently isolated bacilli do not agglutinate well.)	Does not agglutinate with typhoid serum.

10. **Bacillus of Influenza.**—Diagnosis will usually rest upon the morphology and staining peculiarities, described on p. 58, and upon the fact that the bacillus will not grow on ordinary media, but does grow upon hemoglobin-containing media.   It can be grown upon agar-slants which have been smeared with a drop of blood from a puncture in the finger.   Before inoculation these slants should be incubated to make sure of sterility.   The colonies are difficult to see without a hand lens.   They are very minute, discrete, and transparent, resembling small drops of dew.

11. **Bacillus of Tuberculosis.**—The methods of identifying this important organism have been given (pp. 49 and 168).   Cultivation is not resorted to in clinical work.   It grows very slowly and only on certain media. It is Gram-positive and non-motile.

CHAPTER IX

PREPARATION AND USE OF VACCINES

BACTERIAL vaccines, sometimes called "bacterins," which within the past few years have come to play an important rôle in therapeutics, are suspensions of definite numbers of dead bacteria in normal salt solution. While in many cases, notably in gonorrhea and tuberculosis, ready prepared or "stock" vaccines are satisfactory, it is usually desirable and often imperative for best results to use vaccines which are especially prepared for each patient from bacteria which have been freshly isolated from his own lesion. These latter are called "autogenous vaccines." Only through them can one be certain of getting the exact strain of bacterium which is producing the disease.

I. PREPARATION OF VACCINE

The method of preparing autogenous vaccines which is used in the author's laboratory is here described.

1. Preparation of Materials.—A number of 2-ounce wide-mouthed bottles are cleaned and sterilized. Each is charged with 50 c.c. freshly filtered normal salt solution (0.85 per cent. sodium chlorid in distilled water), and is capped with a new rubber nursing-nipple, without holes, inverted as shown in Fig. 162. A small section of hollow wire or a hypodermic needle is thrust through the cap near the edge to serve as an air vent, and the bottle and

contents are sterilized in an autoclave. If an autoclave is not at hand, successive steamings in an Arnold sterilizer will answer, provided it is not opened between steamings. After sterilization, the pieces of wire are pulled out and the holes sealed with collodion.
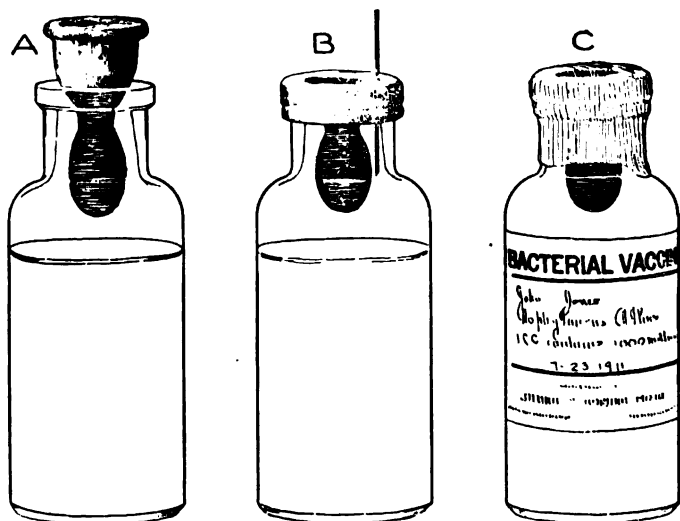
Fig. 162.—Vaccine bottles: A, Cap ready to be applied; B, ready for sterilization; C, finished vaccine.

A number of test-tubes, each charged with 10 c.c. of normal salt solution and plugged with cotton, are also prepared and sterilized.

2. Obtaining the Bacteria.—A culture on some solid medium is made from the patient's lesion, and a pure culture is obtained in the usual way. This preliminary work should be carried through as quickly as possible. If for any reason there is much delay, it is best to begin over again, the experience gained in the first trial en-

abling one to carry the second through more rapidly. When a pure culture is obtained, a number of tubes of blood-serum or agar—10 or 12 in the case of streptococcus or pneumococcus, 4 or 5 in the case of most other organisms—are planted and incubated over night or until a good growth is obtained.

3. Making the Suspension.—The salt solution from one of the 10 c.c. salt-tubes is transferred by means of a
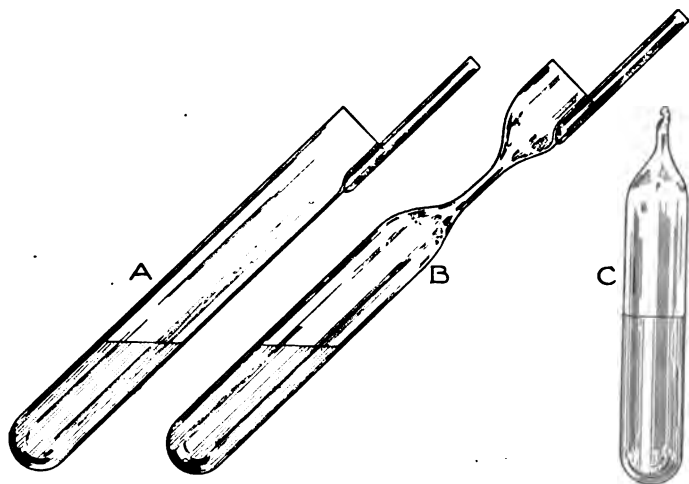
Fig. 163.—Process of making hermetically sealed capsules.

sterile pipet to the culture-tubes, and the growth thoroughly rubbed up with a stiff platinum wire or a glass rod whose tip is bent at right angles. The suspension from the different tubes, usually amounting to about 10 c.c., is then collected in one large tube (size about 6 by $\frac{3}{4}$ inch); and the upper part of the tube is drawn out in the flame of a blast lamp or Bunsen burner, as indicated in Fig. 163, a short section of glass tubing being fused to

SUSPENSION
BLOOD
DILUTING FLUID

The slender portion should be narrower than here represented.
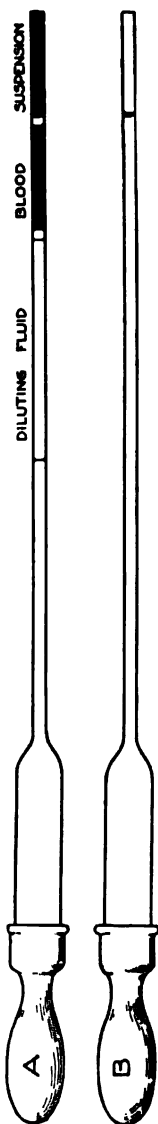
Fig. 164.—Pipets used in counting vaccines by Wright's method.

A

B

the rim of the tube to serve as a handle. It is then stood aside, and when cool the end is sealed off.

The resulting hermetically sealed capsule is next thoroughly shaken for ten to twenty minutes to break up all clumps of bacteria. Some small pieces of glass or a little clean sterile sand may be introduced to assist in this, but with many organisms it is not necessary.

4. Sterilization.—The capsule is placed in a water-bath at 60° C. for forty-five minutes. This can be done in an ordinary rice-cooker, with double lid through which a thermometer is inserted. When both compartments are filled with water it is an easy matter to maintain a uniform temperature by occasional application of a small flame. The time and temperature are important: too little heat will fail to kill the bacteria, and too much will destroy the efficiency of the vaccine.

When sterilization is complete the capsule is opened, a few drops are planted on agar or blood-serum, and the capsule is again sealed.

5. Counting.—When incubation of the plant has shown the suspension to be sterile it is ready for counting.

There must be ready a number of clean slides; a few drops of normal salt solution on a slide or in a watch-glass; a blood-lancet, which can be improvised from a spicule of glass or a pen; and two slender pipets with squarely broken off tips and grease-pencil marks about 2 cm. from the tip (Fig. 164). These are easily made by drawing out a piece of glass tubing, as described on page 398.

It is necessary to work quickly. After thorough shaking, the capsule is opened and a few drops forced out upon a slide. Any remaining clumps of bacteria are broken up with one of the pipets by holding it against and at right angles to the slide, and alternately sucking the fluid in and forcing it out. The pipet is most easily controlled if held in the whole hand with the rubber bulb between the thumb and the side of the index-finger. A finger is then pricked until a drop of blood appears; and into the second pipet are quickly drawn successively: 1 or 2 volumes normal salt solution (or better, a 1 per cent. solution of sodium citrate which prevents coagulation); a small bubble of air; 1 volume of blood; a small bubble of air; and, finally, 1 volume of bacterial suspension. (A "volume" is measured by the distance from the tip of the pipet to the grease-pencil mark.) The contents of the pipet are then forced out upon a slide and thoroughly mixed by sucking in and out, care being taken to avoid bubbles; after which the fluid is distributed to a number of slides and spread as in making blood-smears.

The films are stained with Wright's blood-stain or, better, by a few minutes' application of carbol-thionin, after fixing for a minute in saturated mercuric chlorid solution. With an oil-immersion lens both the red cells

and the bacteria in a number of microscopic fields are counted. The exact number is not important; for convenience 500 red cells may be counted. From the ratio between the number of bacteria and of red cells, it is easy to calculate the number of bacteria· in 1 c.c. of the suspension, it being known that there are 5000 million red corpuscles in a cubic centimeter of normal human blood. If there were twice as many bacteria as red corpuscles in the fields counted, the suspension would contain 10,000 million bacteria per cubic centimeter.

The count can also be made with the hemocytometer, using a weak carbol-fuchsin or gentian violet, *freshly filtered*, as diluting fluid. A very thin cover-glass must be used; and, after filling, the counting-chamber must be set aside for an hour or more to allow the bacteria to settle. Mallory and Wright advise the use of the shallow chamber manufactured by Zeiss for counting blood-plates, but many 2 mm. oil-immersion objectives have sufficient working distance to allow the use of the regular Thoma counting-chamber, provided a very thin cover is used.

6. Diluting.—The amount of the suspension, which, when diluted to 50 c.c., will give the strength desired for the finished vaccine having been determined, this amount of salt solution is withdrawn with a hypodermic syringe from one of the bottles already prepared, and is replaced with an equal amount of suspension. One-tenth c.c. of trikresol or lysol is finally added and the vaccine is ready for use. To prevent possible leakage about the cap, the neck of the bottle is dipped in melted paraffin. The usual strengths are: staphylococcus, 1000 million in 1 c.c.; most other bacteria, 100 million in 1 c.c.

II. METHOD OF USE

Vaccines are administered subcutaneously, usually in the arm or abdominal wall or between the shoulder-blades. The rubber cap is sterilized by filling the concavity with alcohol for some minutes, usually while the syringe is being sterilized. The bottle is then inverted and well shaken, when the needle is plunged through the rubber and the desired quantity withdrawn. The hole seals itself and no collodion is necessary, which is one of the advantages of this form of cap. The most satisfactory syringe is the comparatively inexpensive "Sub-Q Tuberculin" syringe graduated in hundredths of a cubic centimeter.

A rapid and efficient technic for giving the injections is suggested by Major F. T. Woodbury of the Army Medical Corps. The needle is dipped into tincture of iodin and some is drawn into the syringe and expelled; a small quantity of the vaccine or of sterile water is likewise drawn in and expelled, after which the dose to be given is drawn in. The patient's arm is touched with a swab of cotton saturated with tincture of iodin and the injection is made through the resulting brown spot. The syringe is cleaned by drawing into it and expelling, successively, tincture of iodin, alcohol, and air.

III. DOSAGE

Owing to variations, both in virulence of organisms and susceptibility of patients, no definite dosage can be assigned. Each case is a separate problem. Wright's original proposal was to regulate the size and frequency of dose by its effect upon the opsonic index, but this is

beyond the reach of the practitioner. The more widely used "clinical method" consists in beginning with a very small dose and cautiously increasing until the patient shows either improvement or some sign of a "reaction," indicated by headache, malaise, fever, exacerbation of local disease, or inflammatory reaction at the site of injection. The reaction indicates that the dose has been too large. The beginning dose of staphylococcus is about 50 million; the maximum, 1000 million or more. Of most other organisms the beginning dose is 5 million to 10 million; maximum, about 100 million, Ordinarily, injections are given once or twice a week; very small doses may be given every other day.

IV. THERAPEUTIC INDICATIONS

The therapeutic effect of vaccines depends upon their power to stimulate the production of opsonins and other antibacterial substances which enable the body to combat the infecting bacteria. Their especial field is the treatment of subacute and chronic localized infections, in some of which they offer the most effective means of treatment at our command. In most chronic infections the circulation of blood and lymph through the diseased area is very sluggish, so that the antibodies, when formed, cannot readily reach the seat of disease. Ordinary measures which favor circulation in the diseased part should, therefore, accompany the vaccine treatment. Among these may be mentioned incisions and drainage of abscesses, dry cupping, application of heat, Bier's hyperemia, etc., but such measures should not be applied during the twenty-four hours succeeding an injection, since the first effect of the vaccine may be a temporary

lowering of resistance. Vaccines are of little value, and, in general, are contraindicated in very acute infections, particularly in those which are accompanied by much systemic intoxication, for in such cases the power of the tissues to produce antibodies is already taxed to the limit. It is true, nevertheless, that remarkably beneficial results have occasionally followed their use in such acute conditions as malignant endocarditis, but here they should be tried with extreme caution.

Probably best results are obtained in staphylococcus infections, although pneumococcus, streptococcus, and colon bacillus infections usually respond nicely. Among clinical conditions which have been treated successfully with vaccines are furunculosis, acne vulgaris, infected operation-wounds, pyelitis, cystitis, subacute otitis media, osteomyelitis, infections of nasal accessory sinuses, etc. Vaccine treatment of the mixed infection is doubtless an important aid in tuberculosis therapy, and in some cases the result is brilliant. When, as is common, several organisms are present in the sputum, a vaccine is made from each, excepting the tubercle bacillus, of which autogeneous vaccines are not used in practice. To avoid the delay and consequent loss of virulence entailed by study and isolation of the several varieties, many workers make the bacterial suspension directly from the primary cultures. The resulting vaccines contain all strains which are present in the sputum in approximately the same relative numbers. Although open to criticism from a scientific standpoint, this method offers decided practical advantages in many cases.

It has been shown that vaccines are useful in prevent-

ing as well as curing infections. Their value has been especially demonstrated in typhoid fever. Three or four doses of about 50,000,000 typhoid bacilli are given about seven days apart. Results in the army, where the plan has been tried on a large scale, show that such vaccination is effective.

V. TUBERCULINS

Tuberculins contain the products of tubercle bacilli or their ground-up bodies, the latter class being practically vaccines. They are undoubtedly of great value in the treatment of localized tuberculosis, particularly of bones, joints, and glands; and are of rather indefinite though certainly real value in chronic pulmonary tuberculosis, especially when quiescent. The best known are Koch's old tuberculin (T. O.), bouillon filtrate (B. F.), triturate residue (T. R.), and bacillary emulsion (B. E.). There seems to be little difference in the actions of these, although theoretically T. R. should immunize against the bacillus and B. F. against its toxic products. The choice of tuberculin is much less important than the method of administration. The making of autogenous tuberculins is impracticable, hence stock preparations are used in practice.

Since the dose is exceedingly minute, the tuberculin as purchased must be greatly diluted before it is available for use. A convenient plan is to use the rubber-capped 50 c.c. bottles of sterile salt solution described for vaccines (p. 419), adding sufficient tuberculin to give the desired strength, together with 0.1 c.c. trikresol to insure sterility. The practitioner should bear in mind that while tuberculin is capable of good, it is also capable

of great harm. Everything depends upon the dosage and plan of treatment. Probably a safe beginning dose for a pulmonary case is 0.00001 milligram of B. E., B. F., or T. R.; for gland and bone cases, about 0.0001 milligram. O. T. is now used chiefly in diagnosis. The intervals are about one week or, rarely, three days, when very small doses are given. The dose is increased steadily, but with *extreme caution;* and should be diminished or temporarily omitted at the first indication of a "reaction," of which, in general, there are three forms:

(*a*) *General:* Elevation of temperature (often slight), headache, malaise.

(*b*) *Local:* Increase of local symptoms, amount of sputum, etc.

(*c*) *Stick:* Inflammatory reaction at site of injection.

Treatment is usually continued until a maximum dose of 1 mm. is reached, the course extending over a year or more.

VI. TUBERCULIN IN DIAGNOSIS

The tissues of a tuberculous person are sensitized toward tuberculin, and a reaction (see preceding section) occurs when any but the most minute quantity of tuberculin is introduced into the body. Non-tuberculous persons exhibit no such reaction. This is utilized in the diagnosis of obscure forms of tuberculosis, the test being applied in a number of ways:

1. **Hypodermic Injection.**—Koch's old tuberculin is used in successive doses, several days apart, of 0.001, 0.01, and 0.1 mg. A negative result with the largest amount is considered final. The reaction is manifested by fever within eight to twenty hours after the injection. The method involves some danger of lighting up a

latent process, and has been largely displaced by safer methods.

2. **Calmette's Ophthalmo-reaction.**—One or two drops of 0.5 per cent. purified old tuberculin are instilled into one eye. Tuberculin ready prepared for this purpose is on the market. If tuberculosis exists anywhere in the body, a conjunctivitis is induced within twelve to twenty-four hours. This generally subsides within a few days. The method is not without some, though slight, risk of injury to the eye; and the test is absolutely contraindicated in the presence of any form of ocular disease. A second instillation should not be tried in the same eye.

3. **Moro Reaction.**—A 50 per cent. ointment of old tuberculin in lanolin is rubbed into the skin of the abdomen, a piece about the size of a pea being required. Dermatitis, which appears in twenty-four to forty-eight hours, indicates a positive reaction. The ointment can be purchased ready for use.

4. **Von Pirquet's Method.**—This is the most satisfactory of the tuberculin tests. Two small drops of old tuberculin are placed on the skin of the front of the forearm, about 2 inches apart, and the skin is slightly scarified, first at a point midway between them, and then through each of the drops. A convenient scarifier is a piece of heavy platinum wire, the end of which is hammered to a chisel edge. This is held at right angles to the skin, and rotated six to twelve times with just sufficient pressure to remove the epidermis without drawing blood. In about ten minutes the excess of tuberculin is gently wiped away with cotton. No bandage is necessary. A positive reaction is shown by the appearance in

twenty-four to forty-eight hours of a papule with red areola, which contrasts markedly with the small red spot left by the control scarification.

These tests have very great diagnostic value in children, especially those under three years of age, but are often misleading in adults, positive reactions occurring in many apparently healthy individuals. Negative tests are very helpful in deciding against the existence of tuberculosis.

APPENDIX

I. APPARATUS, REAGENTS, AND STAINS

THE apparatus and reagents listed here are sufficient for all but the rarer tests described in the text. Those in smaller type are less frequently required. For ordinary routine work a much smaller list will suffice.

A. APPARATUS

Beakers and flasks, several sizes, preferably of Jena glass.

Blood lancet, or some substitute (Fig. 68).

Bunsen-burner or alcohol lamp.

Buret, 25 c.c. capacity, preferably with Schellbach stripe.

Buret and filter-stand combined.

Centrifuge—hand, electric, or water-power (Figs. 20 and 21). With the last two a speed indicator is desirable. Radius of arm when in motion should be $6\frac{3}{4}$ inches. Plain and graduated tubes accompany the instrument; milk-tubes (Fig. 157) must be purchased separately. The hematocrit attachment (Fig. 77) is not much used.

Cigaret-paper, "Zig-zag" brand or some similar thin paper, for making blood-films.

Corks, preferably of rubber, with one and two holes.

Cover-glasses, No. 2 thickness—$\frac{7}{8}$-inch squares are most convenient.

Cover-glass forceps.

Esbach's tube (Fig. 27).

Evaporating dish.

Filter-paper: ordinary cheap paper for urine filtration; "ashless" quantitative filter-paper for chemic analyses.

Glass funnels.

Glass rods and tubing of sodium glass: for stirring rods, urinary pipets, etc.

Glass slides: the standard 1- by 3-inch size will answer for all work, although a few larger slides will be found convenient; those of medium thickness are preferable.

Graduates, cylindric form, several sizes.

Graniteware basin.

Hemoglobinometer: see pp. 185 to 191 for descriptions of the different instruments.

Hemocytometer: either Türck or Zappert ruling is desirable (Figs. 73, 74, and 79).

Hypodermic syringe: the "Aseptic Sub-Q, Tuberculin," is probably the most useful type.

Incubator (p. 397).

Labels for slides and bottles.

Litmus-paper, red and blue, Squibb's preferred.

Mett's tubes (pp. 300 and 399).

Microscope (Fig. 1). Equipment described on p. 31.

Petri dishes.

Platinum wires (p. 397).

Sterilizers: the Arnold type for steaming; oven for dry sterilization (p. 396).

Stomach-tube.

Test-glass, conic, one side painted half white, half black.

Test-tubes, rack, and cleaning brush.

Ureometer, Doremus-Hinds' pattern (Fig. 24).

Urinometer, preferably Squibb's (Fig. 17).

28

Blood-fixing oven, or Kowarsky's plate (Fig. 84).

Copper-foil and gauze.

Cotton, absorbent, for filtering, etc.

" Cotton-batting " for plugging tubes.

Culture-media.　　The selection depends upon the work to be done (p. 401).

Holt's cream gage and hydrometer (Fig. 156).

Horismascope (Fig. 26).

Pipets, graduated, 5 to 50 c.c. capacity.

Ruhemann's tube for uric-acid estimation (Fig. 25).

Saccharimeter (Fig. 29).

Strauss' separatory funnel for lactic-acid test (Fig. 105).

Suction filter.

Urinopyknometer of Saxe (Fig. 18).

Widal reaction outfit: either living agar cultures of the typhoid bacillus, or the dead cultures with diluting apparatus, which are sold under various trade names.

Water-bath.

B. REAGENTS AND STAINS

All stains and many reagents are best kept in small dropping bottles. Formulæ are given in the text. Dry stains (Grübler's should be specified) and most staining solutions and chemical reagents can be purchased of Bausch & Lomb Optical Co., Rochester, New York, or Eimer & Amend, New York. For the physician who does only a small amount of work, the " Soloid " tablets manufactured by Burroughs, Wellcome & Co. are convenient and satisfactory. These tablets have only to be dissolved in a specified amount of fluid to produce the finished stain. Most of the stains mentioned here come in this form.

Acid, glacial acetic. Other strengths can be made from this as desired.

Acid, hydrochloric, concentrated (contains about 32 per cent. by weight of absolute hydrochloric acid). Other strengths can be made as desired.

Acid, nitric, strong, colorless.

Acid, nitric, yellow. Can be made from colorless acid by adding a splinter of pine, or allowing to stand in sunlight.

Acid, sulphuric, concentrated.

Alcohol, ethyl (grain-alcohol). This is ordinarily about 93 to 95 per cent., and other strengths can be made as desired.

Aqua ammoniæ fortior (sp. gr. 0.9).

Bromin, or Rice's solutions (p. 95), for urea estimation.

Chloroform.

Diluting fluid for erythrocyte count (p. 198).

Diluting fluid for leukocyte count (p. 213).

Dimethyl-amido-azobenzol, 0.5 per cent. alcoholic solution.

Distilled water.

Esbach's or Tsuchiya's reagent (p. 105).

Ether, sulphuric.

Ferric chlorid: saturated aqueous solution and 10 per cent. aqueous solution.

Haines' (or Fehling's or Benedict's) solution (pp. 109, 110).

Lugol's solution (*Liquor Iodi Compositus*, U. S. P.). Gram's iodin solution (p. 57) can be made from this by adding fourteen times its volume of water.

Obermayer's reagent (p. 91).

Phenylhydrazin, pure.

Phenol.

Phenolphthalein, 1 or 0.5 per cent. alcoholic solution.

Purdy's (or Fehling's or Benedict's) solution (pp. 112–115).

Robert's reagent (p. 103).

Sodium chlorid (table salt), saturated aqueous solution.

Sodium hydroxid (caustic soda), 40 per cent. solution; other strengths can be made from this as desired.

Sodium hydroxid, decinormal solution. The practitioner will find it best to purchase this solution ready prepared. Eimer and Amend, New York, and many other chemical supply houses carry it in stock. For ordinary clinical work 41 grams of Merck's "sodium hydrate by alcohol" from a freshly opened bottle may be dissolved in 1000 c.c. water. This makes a normal solution and must be diluted with 9 volumes of water to make the decinormal solution.

Sodium nitrite, 0.5 per cent. solution for diazo-reaction. Must be freshly prepared.

Sulphanilic acid solution for diazo-reaction (p. 128).

Stains:

Carbol fuchsin (p. 50).

Eosin, saturated aqueous solution.

Formalin - gentian - violet, or anilin-gentian - violet (p. 57).

Gabbet's stain or Pappenheim's methylene-blue stain (p. 51).

Löffler's alkaline methylene-blue solution (p. 57).

Pappenheim's pyronin-methyl-green stain (p. 408).

Stain for fat: Sudan III, saturated solution in 70 per cent. alcohol; or 1 per cent. aqueous solution osmic acid.

Wright's or Harlow's stain for blood.

Tincture of guaiac, diluted to a light sherry-wine color (keep in a dark glass bottle).

Turpentine, "ozonized" (p. 125).

Acid, boric, for preserving urine (p. 69).

Acid, oxalic.

Acid, salicylous (salicyl aldehyd), 10 per cent. alcoholic solution.

Alcohol, amylic.

Alcohol, ethyl, absolute.

Alcohol, methyl (pure).

Antiformin (p. 52).

Barium chlorid mixture (p. 89).

Benzol.

Boas' reagent or Günzburg's (p. 290).

Boggs' reagent (p. 387).

Calcium chlorid, 1 per cent. solution.

Canada-balsam in xylol: necessary only when permanent microscopic preparations are made.

Carbon disulphid.

Charcoal, animal.

Chromium trioxid.

Congo-red, strong alcoholic solution.

Copper sulphate.

Diluting fluid for blood-platelet count (pp. 215, 216).

Egg-albumen discs in glycerin (p. 293).

Ether, acetic, pure.

Florence's reagent (p. 393).

Formalin (40 per cent. solution of formaldehyd gas).

India-ink (Günther and Wagner) (p. 391).

Iodin crystals.

Iron sulphid.

Lead acetate (sugar of lead); used in 10 per cent. solution to clarify urine.

Lead acetate, tribasic.

Lime-water.

Müller's fluid saturated with mercuric chlorid (p. 56).

Orcin.

Pepsin, U. S. P.

Phenylhydrazin hydrochlorid.

Potassium ferrocyanid, 10 per cent. solution.

Potassium oxalate (neutral).

Potassium persulphate.

Ruhemann's reagent (p. 97).

Silver nitrate crystals; also dram to the ounce aqueous solution, and " ammoniated " solution (p. 97).

Sodium alizarin sulphonate, 1 per cent. aqueous solution.

Sodium carbonate.

Sodium chlorid, 2 per cent. solution; from this normal salt solution (0.8 per cent.) can be made as desired.

Sodium hyposulphite.

Sodium nitroprussid.

Sodium sulphate.

Stains:

    Bismarck-brown, saturated aqueous or alcoholic solution.

    Carbol-thionin.

    Ehrlich's triple stain for blood.

    Eosin, 0.5 per cent. alcoholic solution for blood.

    Fuchsin, weak solution; can be made when desired by adding a little carbol-fuchsin to a test-tube of water.

    Gentian-violet, saturated alcoholic solution,

    Giemsa's stain (p. 390).

    Methylene-blue and borax solution (p. 254).

    Methylene-blue, saturated aqueous solution for blood.

    Van Gieson's stain for Negri bodies (p. 395).

Sulphur, powdered.

Talc, purified (Talcum Purificatum, U. S. P.).

Trichloracetic acid solution (p. 102).

Uranium nitrate, 5 per cent. aqueous solution.

Xylol.

Zinc, arsenic free.

II. WEIGHTS, MEASURES, ETC., WITH EQUIVALENTS

METRIC

Meter (unit of length):	Millimeter (mm.) = $\frac{1}{1000}$ meter.
	Centimeter (cm.) = $\frac{1}{100}$ meter.
	Kilometer = 1000 meters.
	Micron (μ) = $\frac{1}{1000}$ millimeter.
Gram (unit of weight):	Milligram (mg.) = $\frac{1}{1000}$ gram.
	Kilogram (kilo.) = 1000 meters.
Liter (unit of capacity):	Cubic Centimeter = $\frac{1}{1000}$ liter. Same measure as milliliter (ml.).

1 Millimeter = $\begin{cases} 0.03937 \ (\frac{1}{25} \text{ approx.}) \text{ in.} \\ 1000 \text{ microns.} \end{cases}$

1 Centimeter = $\begin{cases} 0.3937 \ (\frac{2}{5} \text{ approx.}) \text{ in.} \\ 0.0328 \text{ feet.} \end{cases}$

1 Meter = $\begin{cases} 39.37 \text{ in.} \\ 3.28 \text{ feet.} \end{cases}$

1 Micron (μ) = $\begin{cases} \frac{1}{25400} \text{ in.} \\ 0.001 \text{ millimeter.} \end{cases}$

1 Gram = $\begin{cases} 15.43 \text{ grains.} \\ 0.563 \text{ dram} \\ 0.035 \text{ ounce} \\ 0.0022 \text{ pound} \end{cases}$ Avoir.

$\begin{cases} 0.257 \text{ dram} \\ 0.032 \text{ ounce} \\ 0.0027 \text{ pound} \end{cases}$ Apoth.

1 Kilogram = $\begin{cases} 35.27 \text{ ounce (Avoir.).} \\ 2.2 \text{ pound (Avoir.).} \end{cases}$

1 Liter = $\begin{cases} 1.056 \ (1 \text{ approx.}) \text{ quart.} \\ 61.02 \text{ cu. inches.} \\ 1000 \text{ cu. centimeters.} \end{cases}$

1 Sq. Millimeter = 0.00155
1 Sq. Centimeter = 0.1550 } sq. in.
1 Sq. Meter = 1550
1 Sq. Meter = 10.76 sq. feet.

1 Cu. Millimeter = 0.00006 } cu. in.
1 Cu. Centimeter = 0.0610
1 Cu. Centimeter = 0.001 liter.
1 Cu. Meter = $\begin{cases} 35.32 \text{ cu. feet.} \\ 61025.4 \text{ cu. in.} \end{cases}$

1 Inch = 25.399 millimeters.
1 Sq. Inch = 6.451 sq. centimeters.
1 Cu. Inch = 16.387 cu. centimeters.

1 Foot = 30.48 centimeters.
1 Sq. Foot = 0.093 sq. meter.
1 Cu. Foot = 0.028 cu. meter.

AVOIRDUPOIS WEIGHT

1 Ounce = $\begin{cases} 437.5 \text{ grains.} \\ 16 \text{ drams.} \end{cases}$
1 Pound = 16 ounces.

1 Grain = 0.065 ($\frac{1}{15}$ approx.)
1 Dram = 1.77 ($1\frac{3}{4}$ approx.)
1 Ounce = 28.35 (30 approx.)
1 Pound = 453.59 (500 approx.) } grams.
1 Pound = 27.7 cu. inches.
1 Pound = 1.215 lb. Troy.

APOTHECARIES' MEASURE

1 Dram = 60 minims.
1 Ounce = 8 drams.
1 Pint = 16 ounces.
1 Gallon = 8 pints.

1 Dram = 3.70
1 Ounce = 29.57
1 Pint = 473.1 } cu. centimeters.
1 Gallon = 3785.4
1 Gallon = 231 cu. inches.

APOTHECARIES' WEIGHT

1 Scruple = 20 grains.	1 Grain = 0.065 ⎫
1 Dram = 3 scruples = 60 grains.	1 Dram = 3.887 ⎪
1 Ounce = 8 drams = 480 grains.	1 Ounce = 31.10 ⎬ grams.
1 Pound = 12 ounces.	1 Pound = 373.2 ⎭

To convert	minims	into cubic centimeters	multiply by	0.061
" "	fluidounces	" cubic centimeters	" "	29.57
" "	grains	" grams	" "	0.0648
" "	drams	" grams	" "	3.887
" "	cubic centimeters	" minims	" "	16.23
" "	cubic centimeters	" fluidounces	" "	0.0338
" "	grams	" grains	" "	15.432
" "	grams	" drams	" "	0.257

TEMPERATURE

CENTIGRADE.	FAHRENHEIT.	CENTIGRADE.	FAHRENHEIT.
110°	230°	37°	98.6°
100	212	36.5	97.7
95	203	36	96.8
90	194	35.5	95.9
85	185	35	95
80	176	34	93.2
75	167	33	91.4
70	158	32	89.6
65	149	31	87.8
60	140	30	86
55	131	25	77
50	122	20	68
45	113	15	59
44	111.2	10	50
43	109.4	+5	41
42	107.6	0	32
41	105.8	—5	23
40.5	104.9	—10	14
40	104	—15	+5
39.5	103.1	—20	—4
39	102.2		
38.5	101.3	0.54° =	1°
38	100.4	1 =	1.8
37.5	99.5	2 =	3.6
		2.5 =	4.5

To convert Fahrenheit into Centigrade, subtract 32 and multiply by 0.555.

To convert Centigrade into Fahrenheit, multiply by 1.8 and add 32.

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